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MOTIVATIONAL EFFECTS OF PARAMETRIC MANIPULATIONS OF ELECTRICAL BRAIN STIMULATION OF THE RAT LATERAL HYPOTHALAMUS

RONALD WILLIAM SKELTON

A Thesis

in

The Department

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ABSTRACT

MOTIVATIONAL EFFECTS OF PARAMETRIC MANIPULATIONS OF ELECTRICAL BRAIN STIMULATION OF THE RAT LATERAL HYPOTHALAMUS

RONALD WILLIAM SKELTON

Rats were trained to turn on and turn off electrical brain stimulation delivered through electrodes in the lateral hypothalamus. Parametric manipulations of the current intensity and temporal parameters of the ESB differentially shifted the frequency thresholds of these two behaviours. Shifts produced by changes in the current intensity are shown to reveal differences in the spatial distributions of directly activated neuron populations relevant to each behaviour. Shifts produced by pulse-pair stimulation at the intrapulse interval of 0.2 msec are argued to be due to the effects of local potential summation (LPS). Since LPS effects are due to the recruitment of neurons beyond the fringe of the stimulation field and since the shifts were different for the two behaviours, it is argued that these shifts also reveal differences in the spatial distributions of the relevant neurons.

When the intrapulse interval was between 0.4 and 5.0 msec, the shifts were systematically related to the interval and resembled the post-stimulation excitability cycle of populations of neurons. Unfortunately the variability of these shifts was so large as to mask any minor differences between the two behaviours that might have been caused by differences in the refractory periods of the relevant neruon populations. Because the data indicate that the range of refractory periods within the neuron populations underlying each behaviour are large enough to encompass many

different kinds of neurons, it is argued that the data do not necessarily show that there is one population responsible for both behaviours. Indeed, three other interrelated lines of evidence all indicate that the neuron populations responsible for each behaviour are functionally distinct.

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I would like to thank Peter Shizgal not only for his supervision of this thesis, but also for his contributions to my education over the past two years. Largely through his efforts, I became acquainted with many new facets of psychological research and was encouraged to refine and systematise many skills which I had been using naively or intuitively. Peter was a model of excellence which I could only emulate. His influence extended from the technical details of the research, through the processes of logical thought required for the formulation of the experimental objectives to the generation and analysis of the data and the effective communication of our findings and ideas. I am extremely grateful to Peter for all the time and energy that he expended on my behalf.

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table that I allowed and it is only through her efforts and editorial supervision that this thesis is in its present form. To her I extend my deepest gratitude.

R.W. Skelton

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Introduction

Electrical brain stimulation (ESB) is a potentially powerful tool in the search for relationships between neural activity and behaviour. It is usually inferred that a behavioural change that is contiguous with the onset, duration or offset of the ESB is a consequence of the activity of the neural structures in the vicinity of the electrode tip. Unfortunately, this inference is much less straightforward when the ESB causes more than one behavioural change. One example of this is the ESB of certain areas of the lateral hypothalamus (LH). Animals will readily learn to seek ESB of these areas but will also readily learn to escape it when it is prolonged. If given the opportunity, animals will repeatedly turn it on, then off, on then off and so on.

Several theories have evolved to explain this paradoxical phenomenon that has been observed in several different regions of the brain. These theories can be roughly categorized as one-affect theories or two-affect theories and can be further subdivided on the basis of their proposed neural mechanisms. The simplest one-affect theory proposes that the onset of the ESB is positively reinforcing but that the system mediating the effect adapts to the ESB when it persists. According to this hypothesis, in order to receive the positive reinforcement again, the animal must first turn the stimulation off, and then turn it back on again. In other words, the act of turning the stimulation off (the OFF response) is chained to the act of turning it on (the ON response). A second one-affect theory proposes that both the onset and the offset of the ESB are

positively reinforcing. Two affect theories generally propose that initially the ESB is positively reinforcing but when it persists, it becomes negatively reinforcing. Some of the theories propose that this change in the reinforcement-value of the ESB is a consequence of the prolonged activation of a single population of neurons while other theories postulate the simultaneous activation of two or more distinct populations.[1]

Each of these theories makes a different inference about the relationship between the activity in the region of the electrode tip and the behaviour of the animals. Some of the theories imply that the activity is similar to normal activity, some imply that the pattern of the activity is abnormal and some imply that the activity in any one population is similar to normal activity but that the simultaneous activity of two or more populations may not necessarily be a part of normal brain functions. If it can be established that one of these theories is more likely to represent the course of events which occur during the ESB, then this will augment the usefulness of ESB in studying the neural basis of affect.

Selected Review of the Literature

The one-affect, adaptation hypothesis, presently championed

[1] Throughout this thesis, when the consequences of the ESB are phrased in terms of its effect on a "population", the population will refer to those functionally related, behaviourally relevant neural elements in the vicinity of the electrode tip whose activity is altered by the stimulating current directly. Other neuronal changes which result from this change in activity will be refered to as changes in "systems".

by Deutsch, was originally proposed by Stein (1962) in an effort to account for the behaviour of two animals with septal electrodes. Unlike the eight rats with hypothalamic and mid-brain electrode placements that selected shorter stimulation durations as the current intensity was increased, the preferred duration of these two variant animals increased with current intensity. Stein proposed that these two animals who had septal electrodes were holding down the lever until the stimulation was no longer rewarding. Because the behaviour of these animals was different from his other rats and different from the behaviour of other rats with electrodes implanted outside the septum (Bower and Miller, 1958), he proposed that the effect was site specific.

Although the two succeeding studies on the preferred duration of septal ESB failed to replicate these results (Valenstein and Valenstein, 1964, and Atrens, 1970), Deutsch and Hawkins (1972) revived this hypothesis to account for the behaviour of their animals in a lever-choice situation. They found that rats receiving prolonged stimulation of the lateral hypothalamus (LH) preferred a brief increase in stimulation frequency prior to stimulation offset over an immediate termination of stimulation. They felt that the increase in frequency was capable of momentarily overcoming the adaptation and was, therefore, rewarding. They reasoned that animals would not choose to increase aversive stimulation.[2] In a later paper, Deutsch (1973) did

[2] Phrases that imply such psychological constructs as reward or punishment will be used only to reveal how other authors have expressed their ideas. This thesis makes no attempt to determine what the animal "feels" when the ESB is on. The effects of ESB

admit that these results could be explained by a two system hypothesis if it could be shown that the aversive effect of the stimulation lagged behind the appetative effect in time. A temporary overbalancing of the aversive effect might then result from an increase in stimulation frequency. Subsequent experiments (Shizgal, 1975; Shizgal and Matthews, 1977) have examined this possibility.

By measuring changes in the current thresholds for turning LH ESB on and off in a lever-shuttle box, Shizgal (1975) was able to show that the ON system responded promptly to the stimulation (within one or two seconds) while the OFF system required prolonged stimulation (more than 3 seconds) in order to achieve an asymptotic response. Part of this finding was to some degree replicated by Deutsch himself. Deutsch, Roll and Wetter (1976) found that rats lever-pressing for different durations of ESB would not discriminate between duration of stimulation longer than one second. Although Deutsch et. al. interpreted their results in terms of adaptation of the reward system, Shizgal gave very persuasive arguments for his interpretation of his results in terms of integration characteristics of the ON and OFF systems. These arguments will be further detailed in discussions of other theories, to which they are more relevant. Deutsch, however, had another line of evidence consistent with the adaptation hypothesis.

will be defined operationally. Stimuli that animals seek will be termed appetative and stimuli they avoid or escape will be termed aversive. The appetative effects of the ESB will be considered to be due to the activation of the "ON system" while the aversive effect will be considered to be due to the activation of the "OFF system". These two systems may be one and the same system.

Using a modification of a pulse-pair technique that Deutsch developed in 1964, Deutsch and Albertson (1974) estimated the refactory period (RP) of fibers mediating lever pressing for brief trains of ESB and also for a decrease in frequency of continuous ESB. They found that trains of pulse-pairs could be discriminated from trains of single pulses when the interval between the two pulses of the pairs was equal to or greater than 0.6 msec., regardless of the behaviour. They argued that because the fibers mediating both behaviours were unable to respond to the second pulse of the pair for the same length of time (0.6 msec.) it was likely that the same set of fibers was responsible for both behaviours.

Until we have some estimate of the variability in RP's for different neurons involved in motivation it is impossible to assess the likelihood of two different neuronal populations manifesting similar RP's. Thus, the Deutsch and Albertson study is not definitive. Furthermore, the validity of the RP estimate of this and all other studies that have used a single frequency and intensity to reveal RP's has been seriously questioned by a student of Deutsch's (Yeomans, 1975). Briefly, it was Yeomans' contention that a behavioural rating that could jump from floor to ceiling with a 50% shift in intensity or frequency of ESB is incapable of accurately describing the post-stimulation excitability cycle of neural elements. This present study is, in many ways, a replication of Deutsch and Alberston's work, using the more accurate frequency scaling technique that Yeomans proposed. The necessity of Yeomans' scaling technique is illustrated by the fact Deutsch and Albertson had to discard one animal's data

because the pulse-pair stimulation was always preferred regardless of the interval between the first and second pulses.

A more direct test of the adaptation hypothesis was conducted by Mendelson and Freed (1973). Rats were trained to shuttle from one side of a box to the other in order to turn LH ESB on and off. They reasoned that rats who were turning the stimulation off only so they could turn it back on again would choose to leave the stimulation on longer if the opportunity to turn the stimulation on again was removed. They found that when the rats had to wait 24 hours between stimulations, they turned the stimulation off just as quickly as they had before. This finding is very difficult to reconcile with the pure adaptation hypothesis but might be expected from the one affect hypothesis which states that the onset and offset of the ESB are positively reinforcing (Hodos, 1965).

One of the weaknesses of all one-affect hypotheses in general is the discrepancy between the behaviour of naive subjects that would be predicted by these hypotheses and the behaviour that is actually observed. Initially, the stimulation usually results in a brief pause followed by exploratory behaviour. As the stimulation persists many rats begin to search frantically about and often darting, jumping, defacating and vocalizing ensue. Well-trained animals who are given the opportunity to turn the stimulation off, usually do so in an orderly and well-directed manner. While it is conceivable that animals could frantically seek the chained or the primary positive reinforcement of the ESB offset, it would be expected that this behaviour would only appear once the animals were well-trained

in the experimental situation. Furthermore, this behaviour should resemble the appetitive pattern observed during the time that stimulation is off and the untrainted rat is "searching" for the bar. It does not. This inconsistancy between the actual behaviours observed and the expectations given by one-affect hypotheses naturally leads to the consideration of two-affect hypotheses.

These hypotheses propose that the initial effect of the ESB is positively reinforcing but when the ESB persists, it becomes negatively reinforcing. The many different mechanisms that have been proposed generally involve the direct activation of either one or two populations of neurons that are functionally distinct but anatomically overlapping with each population responsible for one of the affects. (For early references see Roberts, 1958b; Bower and Miller, 1958; Stein 1962; Olds and Olds, 1963; or Margules, 1966). The paradox that the animals seek and escape the stimulation and yet do not avoid it (eg. Roberts, 1958a; Bower and Miller, 1958) is generally attributed to the brisk response of the ON system to the ESB and a sluggish response in the OFF system (see Shizgal, 1975; for an excellent summary).

One system hypotheses postulate that this difference in responsiveness of the two systems could occur "downstream" from the stimulation site. Examples of these mechanisms include overexcitation or fatigue (Roberts, 1958b), an activation-inhibition sequence (Grastyan, Czopf, Angyan and Szabo, 1964) or different effects from different axonal collaterals (Szabo, 1973). The importance of being able to distinguish between these different one-population alternatives depends upon the necessity of rejecting the simpler two-population hypothesis. Although

most of the studies on the aversive and appetative effects of ESB have concentrated on the one or two affect question, the data from several are applicable to the one versus two populations question.

Brodie, Malis, Moreno and Boren (1960) found that stimulating the medial forebrain bundle (MFB) in monkeys for up to 60 minutes elicited no signs of aversion although the stimulation was clearly appetitive. This implies that the frequent observation of both appetitive and aversive effects from diverse electrode locations (eg. Valenstein and Valenstein, 1964) may be largely a consequence of the stimulation of the small brain of the rat with relatively larger electrodes. When Margules (1966) carefully varied the current intensity of ESB in several locations within the LH, he found large differences in the way animals responded to the intensity changes. Some animals in his two lever-shuttle box would turn the stimulation off at lower intensities than they would turn it on. Some animals would turn it on at lower intensities than they would turn it off. Some animals had intensity thresholds that were approximately equal for the two behaviours and some animals would only turn the stimulation either on or off at all intensities. Margules concluded that because current spread increased with current intensity, the relationship between the intensity thresholds for the two behaviours reflected the spatial relationship between the electrode tip and the neural systems responsible for the positive and negative reinforcement.

This difference in responsiveness of the two behaviours to current manipulations was also shown by van Sommers and Teitelbaum (1973). They also found that the relative intensity thresholds of the two behaviours depended upon the location of the electrodes. They

also showed that the time course and severity of the deficits caused by a lesion through the stimulating electrode were different for turning on and turning off the ESB. They too concluded that the spatial distribution of neural elements responsible for positive reinforcement was different than the spatial distribution of those responsible for negative reinforcement. Given these studies, it appears likely that the appetative and aversive effects of LH ESB are due to the concurrent activiation of distinct neural populations.

This finding to some extent reduces the power of ESB to probe the behavioural functions of neuroanatomical areas. Research into brain-behaviour relationships would be much easier if neural structures were organized along the lines of psychological constructs. Fortunately, there may be ways to circumvent the problems introduced by the spatial intermingling of neuron populations relevant to divergent behavioural phenomena. A recent study by Shizgal and Matthews (1977) has shown that the balance of different behavioural effects seen following ESB through a single electrode can be shifted by manipulations of the temporal parameters of the stimulation.

They gave strong evidence that chopping the trains of ESB up into short bursts with short interburst intervals had little effect on the latencies to turn the stimulation on, but increased the latencies to turn the stimulation off to such a degree that in many instances the rats never turned the stimulation off within 20 seconds. They reasoned that the ON system which seemed to respond briskly and briefly to the stimulation, was little affected by breaking the stimulation up, but the sluggish OFF system which required several seconds of stimulation to reach a level sufficiently high to affect behaviour, was severely

affected. In this manner they were able to illustrate how behavioural systems that appear to have a great deal of neuroanatomical overlap might be studied independantly.

A second way to study different behavioural effects of individual electrode locations is through neurochemical interventions. Atrens, von Vietinghoff-Reisch, Der Karabetian and Masliyah (1974) were able to show differential effects of amphetamine on turning on and turning off LH ESB in a shuttle box. The direction of the changes induced by the amphetamine depended upon the location of their electrodes within the LH. One implication of this study is that a change in rate of responding for brief trains of ESB could as easily be due to an attenuation of the aversive effects as a facilitation of the rewarding ones. Although this point was made by Margules in 1966, it has been largely ignored in many investigations of the neural basis of "brain stimulation reward" (see Waquier and Rolls, 1976). It is noteworthy that Hoebel(Note 1) uses his animals' turning off behaviour as a control for performance deficits when he uses an experimental manipulation that decreases their turning on of ESB. This ability to distinguish behavioural effects of ESB on the basis of the neurochemical requirements becomes increasingly important when recent advances in histochemical techniques are considered (eg. Battenburg and Bloom, 1975). Because the brain has such small components that have such complex interactions, it is important to label as many characteristics of the neural systems involved in particular behaviours as possible.

An additional manner in which neurons can be differentiated is on the basis of their neurophysiological properties. For example, all neurons have a post-stimulation excitability cycle. Once an area of

the neuronal membrane has propagated an action potential, there is a brief period during which the membrane is incapable of propogating another action potential. This is called the absolute refractory period (ARP). Following this there is a relative refractory period (RRP) during which the membrane is hypoexcitable[3] (eg. Erlanger and Gasser, 1937). The duration of the refractory periods are relatively stable within any particular neuron but vary considerably between different types of neurons. RP's can not only be used to distinguish neurons, but they can also indicate the size of the axon in which they occur (Hursch, 1939). If the RP's of the directly stimulated elements that subserve the behavioural effect of ESB can be established, then it might be possible to pick out relevant structures histologically or neurophysiologically from other structures in areas where there are a large variety of neurons.

It has already been noted that Deutsch (1964) was the first to apply this approach to ESB and that he and others (Deutsch and Albertson, 1974; Schmitt, Sandner and Karli, 1976) have applied it to the investigation of the turning on and turning off of LH ESB. Yeomans (1975) has questioned the accuracy of the estimates derived from the techniques that these studies (as well as many others) have employed, and so has cast doubt upon the experimental conclusions. This study is another attempt to estimate the RP's of the directly stimulated

3 In some neurons, under appropriate conditions, a supernormal period (SNP) and a subnormal period follow the relative refractory period. These two stages are labile and depend on the state of fatigue of the axon.

elements responsible for the appetative and aversive effects of the LH ESB. It is necessary, however, to first reveal the procedures and faults of previous studies and show how Yeomans' new procedure overcomes these faults.

The basic unit in the physiological study of post-stimulation excitability cycle is the pulse-pair. A conditioning pulse (C-pulse) is used to fire the axon. The second pulse of the pair, the T-pulse tests the membrane for excitability. In the original studies (see Erlanger and Gasser, 1937) the interval between the two pulses (the C-T interval) was held constant and the intensity of the T-pulse increased until a second axonal firing was elicited. The ratio, T-pulse threshold intensity to the C-pulse threshold intensity, was used as the indicator of membrane excitability. This procedure had to be modified for behavioural tests.

Because only two pulses of ESB are not likely to be adequate as reinforcement, pulses must be delivered in longer trains. Deutsch (1964) was the first to deduce that a train of C-and T-pulses with very short C-T intervals, such that T-pulses were delivered during the neurons' RP, would cause the same behavioural effect as trains of C-pulses alone. He delivered various trains of ESB, holding the frequency and intensity constant but varying the C-T interval. He concluded that the C-T interval beyond which the train of pulse pairs was more effective than the train of single pulses represented the RP of the behaviourally relevant neurons directly stimulated by the ESB. The RP estimates that he presented were reasonably consistant with this hypothesis.

In 1973, however, Gallistel pointed out that the effect of adding T-pulses at C-T intervals greater than the RP's of the relevant

neurons should have the same effect as doubling the frequency of the pulses in the train. Yeomans in 1975 questioned the ability of most behavioural scales to consistantly reveal the full effects of a doubling of stimulation frequency and, therefore, questioned the accuracy of previous RP estimates that were based on behavioural scales. He showed for example that a 25-50% increase in ESB frequency was often capable of shifting self-stimulation response rates from below operant threshold to asymptotic levels (a shift of 0-100% on the behavioural scale). This meant that some of the effects of the T-pulses could easily be masked or distorted by floor or ceiling effects. Yeomans argued that accurate estimates of the effects of the T-pulses could only be derived from measures of the behavioural consequences of the ESB that were free from floor and ceiling effects. One category of measures which meets this requirement is constant output functions.

Constant output functions reveal the change in one parameter required to offset the effects of a change in another parameter of the ESB in order to maintain a constant level of behaviour. If the behaviour is monotonically related to both of the parameters being changed and if the constant level of behaviour being studied is above the "floor" and below the "ceiling" of the behavioural response, then the inference can be made that all the pairs of the values of the two parameters that produce the same level of behaviour also produce the same excitation in the relevant neural systems.[4]

[4] A discussion of one constant output function, the trade-off between ESB frequency and intensity, is contained in the Results and Discussion. A full discussion of constant output functions and related issues can be found in Edmonds, Stellar and Galistel, 1974; Yeomans, 1975 and Shizgal, 1975.

Yeomans' constant output (threshold responding) procedure was designed to estimate the effectiveness of the T-pulse relative to the effectiveness of the C-pulse at each C-T interval. When the C-T interval is greater than all the RP's of the relevant neurons, the T-pulses have the same effect on behaviour as the C-pulses. In other words, the relative effectiveness of the T-pulses (as compared to the effectiveness of the C-pulses) is 1.0. When the C-T interval is shorter than the RP's of all the neurons, the T-pulses have no effect upon the behaviour and so the relative T-pulse effectiveness (T_E) equals zero. When the C-T interval is long enough to exceed some but not all of the RP's of the relevant neurons, the T-pulses fire some but not all of the neurons fired by the C-pulses. Therefore, the behavioural effect of the T-pulses is less than the behavioural effect of the C-pulses and so the T_E at that C-T interval will be somewhere between zero and 1.0.

The calculation of T_E from the rat's frequency thresholds (FT) required a few assumptions. The first is that when brief pulses (.1 msec) of a fixed current intensity and with long C-C intervals (minimum, 10 msec) are used, all C-pulses fire all the neurons in a constant field around the electrode tip once. The second assumption is at the heart of constant output functions. Since the behaviour that Yeomans was studying (self-stimulation) was monotonically related to his scaling parameter (frequency), then two frequencies which produced the same behaviour could be assumed to be producing the same amount of relevant neural excitation. The third assumption was that for brief trains of ESB, the excitation produced by a train of stimulation equals the

product of the number of neurons fired times the number of times they are fired.[5]

Therefore, the excitation produced by a train of single pulses = (no. of neurons fired by C-pulses)(no. of C-pulses). Similarly the excitation produced by a train of double pulses = (no. of neurons fired by C-pulses)(no. of C-pulses) + (no. of neurons fired by T-pulses)(no. of T-pulses). Because the behavioural output of an animal pressing for his FT is a constant, the excitation produced by the animals FT for single pulses (FT_{SP}) equals the excitation produced by the animals FT for double pulses (FT_{DP}). Since the number of C-pulses in any train of stimulation equals the product of the train duration and the frequency of the stimulation, and since the number of T-pulses in a train of double pulses equals the FT_{DP} times the train duration, by measuring FT_{SP} and FT_{DP} and holding train duration constant, we can equate the two previous statements and write:

(no. of neurons fired by C-pulses)(FT_{SP} X train duration) = (no. of neurons fired by C-pulses)(FT_{DP} X train duration) + (no. of neurons fired by T-pulses)(FT_{DP} X train duration).

The relative behavioural effect of the T-pulses (T_E) will depend upon the ratio of the number of neurons fired by the T-pulses to the number of neurons fired by the C-pulses. To determine how this ratio can be calculated from the FT's, first divide both sides of the equation by

[5] The justification of all these assumptions are discussed in the Results and Discussion section and in Edmonds, Stellar and Gallistel, 1974; Yeomans, 1975; Matthews, 1975 and Shizgal, 1975.

the train duration and then combine the two expressions containing (no. of C-pulses) to form the following equation:

(no. of neurons fired by T-pulses)(FT_{DP}) = (no. of neurons fired by C-pulses)(FT_{SP}) - (no. of neurons fired by C-pulses)(FT_{DP}).

By dividing both sides of the equation by (no. of neurons fired by C-pulses) and by FT_{DP}, we get:

$$\frac{\text{(no. of neurons fired by T-pulses)}}{\text{(no. of neurons fired by C-pulses)}} = \frac{FT_{SP} - FT_{DP}}{FT_{DP}}$$

Therefore:

$$T_E = \frac{FT_{SP} - FT_{DP}}{FT_{DP}}$$

or

$$T_E = \frac{FT_{SP}}{FT_{DP}} - 1$$

It is possible to get an intuitive appreciation of the meaning of this ratio: when the T-pulses are as effective as the C-pulses (because they fire all the same neurons), the FT_{DP} should be 1/2 the FT_{SP}, because there are twice as many pulses per second in a double pulse train. At such a C-T interval: $FT_{DP} = 1/2\ FT_{SP}$

and so, $T_E = \frac{FT_{SP}}{\frac{1}{2}\ FT_{SP}} - 1 = 1.0$

When the C-T interval exceeds the RP's of some of the neurons, the FT_{DP} will be proportionally smaller than the FT_{SP} and so T_E will lie between 0.0 and 1.0.

Throughout this presentation it was implicitly assumed that the behavioural weight of all the relevant neurons are equal. Because

of the "size principle", that is not necessarily so. Large neurons may have a greater behavioural weight than small neurons (Henneman, Somjen and Carpenter, 1965; Davis, 1971). The value of T_E at any given C-T interval, therefore, does not represent the proportion of the relevant neuron population whose RP's are exceeded but rather represents the proportional behavioural contribution of those relevant neurons whose RP's are exceeded (Yeomans, 1975; Hawkins, 1976).

Up to this point, the phrase "C-T interval which exceeds the RP" has not defined whether the absolute refractory period (ARP) or the relative refractory period (RRP) must be exceeded by the C-T interval before the T-pulse can fire the neuron. The answer to this question depends upon the spatial relationship between the particular neuron and the electrode tip. Because local current density decreases as the square of the distance from the electrode (see the Results and Discussion, Matthews, 1975, or Ranck, 1975), those neurons near the electrode tip "see" a much higher current than those further away. Neurons close to the electrode tip will, therefore, be fired by the T-pulse when the C-T interval is marginally greater than the ARP. Those neurons which lie just inside the fringe of the C-pulse stimulated field receive stimulation that is just barely above their threshold. These neurons will, therefore, be fired by the T-pulse only when the C-T interval is longer than their RRP.

Yeomans (1977) used an ingenious procedure involving unequal pulses to estimate the behavioural contribution of those neurons which do not fire until the C-T interval exceeds the RRP. Because he found that the relative contribution of these neurons was very slight, he concluded that the curve relating T_E to C-T interval determined by the ratio of the FT's primarily represent the effects of the distribution

of ARP's.

In this same study Yeomans (1977) also determined the T_E versus C-T interval curve for self-stimulation from a non-MFB placement (near the Locus Coeruleus). He found the curves to be indistinguishable from those obtained by the same method for MFB placements. In another study (Dennis, Yeomans & Deutsch, 1976) the same frequency scaling procedure was used to study the RP's of neurons in the medial lemniscus that were involved in operant escape from ESB. These authors were able to discriminate three different neuron populations by their RP's.

Experimental Objectives

If the RP's of the neurons whose direct excitation leads to the turning on and turning off of ESB, in the 2-lever shuttle task, could be shown to be distinguishable by frequency scaling methods, then the two-affect, two population hypothesis would be strengthened still further. If the RP's for the ON and OFF populations could be accurately described, it is conceivable that this information would be useful to electrophysiological experiments aimed at determining neuronal interactions which contribute to the aversive and appetative effects of LH ESB.

In addition to the attempts to estimate and discriminate RP's, other parametric manipulations of the ESB were used to determine certain other events local to the electrode tip and also to determine in what manner the current intensity (I) and the frequency (F) of the ESB interact to produce a particular behavioural output. The behavioural measures were the latencies of the responses which turned the ESB on and off. This paradigm was used not only because it allowed the simultaneous determination of two separate effects of the stimulation, but also because it enabled the ESB to be automatically started and/or stopped

in the absence of one or both of the responses. In this respect, the occurance of both appetitive and aversive effects was not required to evaluate the magnitude of either one of them.

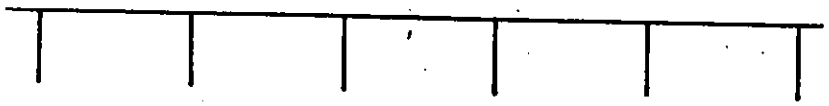
Although the magnitude of the effects were evaluated by the latencies to turn the ESB on or off, the important scalar was always the frequency at which the latency of each response exceeded some fairly large value (15 seconds). In other words, an estimate of the frequency that resulted in a constant behavioural output. The effect of changes in I or the temporal pattern of the stimulation were thus scaled by the change produced in the frequency necessary to produce this constant behavioural output.

Throughout this thesis, frequency is always presented in terms of the period (P) of the stimulation. P is the inverse of frequency and represents the interval between the start of one cycle of stimulation and the start of the next. Period is used because it is more consistantly applicable to a variety of waveforms and because it was the P of the stimulation that was actually programmed and then monitored on the oscilliscope. The relationship between frequency, period and C-T interval are illustrated in Figure 1. It is important to remember that as P increases, the number of pulses (and hence the total charge) delivered decreases if all other parameters (intensity, pulse duration, and train duration) are held constant.

The rationale for measuring the P that produces a constant behavioural output will be developed more completely in the Results and Discussion section, but a brief explanation might temporarily justify the time-consuming procedure that was used. At the present

Figure 1. A graphic representation of the relationship between period (P), frequency (F) and the interval between two pulses of a pair (C-T interval). Note how the frequency of the pulses in the double pulse condition (F_{DP}) becomes ambiguous. Also note how the number of single pulses within a fixed duration decreases as P increases.

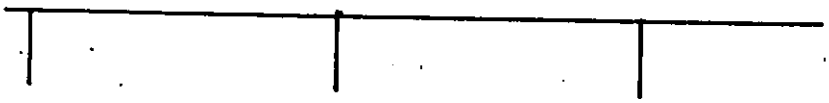
"single pulses"



P = 10 msec

F = 100 Hz

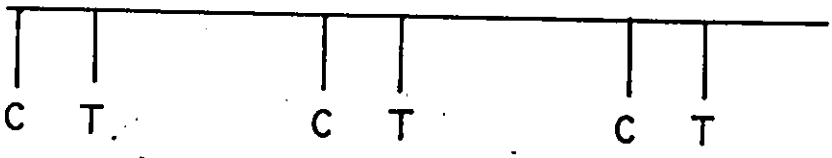
"single pulses"



P = 20 msec

F = 50 Hz

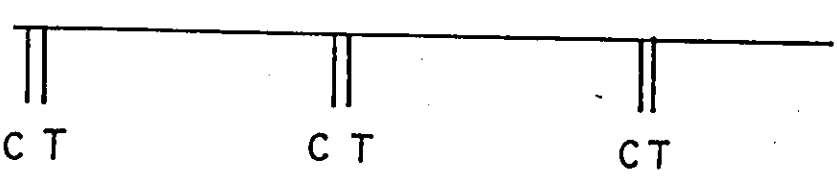
"double pulses" C-T = 5.0 msec



P = 20 msec

F = 50 Hz or 100 Hz ?

"double pulses" C-T = 1.0 msec



P = 20 msec

F = 50 Hz or 100 Hz ?

10 msec

time, the process by which electrical stimulation is translated into neural activity is not completely understood. The process which translates this neural activity into behavior is still largely a mystery.

Once the assumption is made, however, that a particular behaviour is related to activity in a particular "system" in the brain, then it is a reasonable assumption that a particular level of that behaviour is due to a particular level of activity in the responsible "system". It is a much more tenuous assumption that the behaviour is related linearly to the neural activity. For example, a one hundred percent increase in bar press rate might not represent a 100 percent increase in neural activity.

In the present experiment, the primary concern is whether the system subserving the turning off of the ESB is the same as the system subserving the ON-response. The basic method for determining the possible independance of the neural populations was the evaluation of the effects of changes in the ESB parameters. If the function which translates the neural activity into behaviour is different for the two behaviours, then changing the activity in one system responsible for both behaviours would show differential effects upon the two behaviours. Although this would establish the independance of the two systems at some point in the brain, it would not establish whether this independance existed in the region of the electrode tip.

The objective of the present study, therefore, was to examine some effects of changes in ESB which are most likely local to the electrode tip and to use a measure that does not require knowledge of the function that translates neural activity into behaviour. The estimate of T-pulse effectiveness by comparing P (or frequency) thresholds is a

constant output function that depends almost completely upon the excitability characteristics of the neurons directly stimulated by the ESB. The use of P thresholds to evaluate the effects of changing the I of the ESB will be shown to be a procedure which reveals certain characteristics of the spatial distribution of the relevant neuron populations in the region of the electrode tip. Although the determination of these constant-output functions was very time-consuming and limited the number of animals that could be tested, the benefits of their use were felt to outweigh these constraints.

Methods

Animals and electrodes

Under nembutal anaesthesia, twenty-two male Sprague-Dawley rats (Canadian Breeding Farms and Laboratories) weighing between 350 and 400 gms were implanted with two or four electrodes. The electrodes were constructed from 00 stainless steel insect pins coated with Formvar (Universal Wire Corporation) except for .4-.5 mm of the pointed tip. The pins were soldered to a 9-pin connector (I.T.T. Cannon Electronics, MD1-9PL1) in a single plane such that the outermost electrodes were four mm apart and the two inner electrodes (when present), placed 1.2 mm apart, were evenly spaced between the outer two. The two inner electrodes (when present), were 1.0 mm longer than the two outer electrodes. The entire assembly was mounted on a Kopf stereotaxic instrument so that all the electrodes were in the same vertical and coronal plane with respect to the animal. The outer electrodes were sunk between 7.5 - 8.0 mm below the surface of the cortex, 6.7 mm anterior to ear bar zero while the dorsal surface of the frontal bones was level between Bregma and Lambda.

After the completion of all experimental testing, the animals were killed with an overdose of nembutal and were then perfused with saline followed by 10% formalin. The brains were removed and after at least a week of storage in the formalin, cut into 5.0 mm slabs, frozen on dry ice and sectioned in a freezing microtome. Serial 40 micron sections containing the electrode tracts were stained with thionin and then the location of the electrode tips were traced onto plates

from the Konig and Klippel atlas of the rats brain using a microprojector. All outer electrode locations were found to be in or near the Medial Forebrain Bundle in the LH while the inner electrodes (when present), were in the ventromedial hypothalamus.

Test Environment

The animals were tested in a clear plexiglas box 25 cm x 25 cm x 75 cm high, ventilated through the top and bottom. The floor was constructed of stainless steel bars. The test chamber had two Compound Rodent Levers (BRS/LVE) mounted 3 cm off the floor in opposite corners of the box, 2 cm from the front or back wall. In front of each of the levers was a plexiglas obstruction that eliminated most lever depressions due to movements other than paw- or snout-presses.

The test chamber was in a sound attenuated room isolated from the electronic equipment and the movements of the experimenters. The behaviour of all but the first two of the experimental animals was observed via continuous close circuit television. Efforts were made to limit unnecessary noises and to keep all necessary noises constant or random with respect to the stimulation.

Behavioural Assessment and ESB Parameters

When the animals pressed one lever (the ON lever), a twenty second train of ESB was started. The animal could press the other lever (the OFF lever) to stop the ESB for twenty seconds. Depression of the ON lever would only affect the ESB when it was off and depression of the OFF lever would only affect the ESB when it was on. When neither lever was pressed during the appropriate phase, the ESB was

delivered in a 20-sec on, 20-sec off pattern. The behavioural measures were the animal's ON Latency (the average time taken to turn the ESB on, once it went off) and the animal's OFF Latency (the average time taken to turn the ESB off, once it went on).

The electrical stimulation consisted of 0.1 msec cathodal rectangular pulses generated by a constant current amplifier (Mundl, see Reference Note 2). The amplifier had high output impedance during the pulse but only a 1K Ω output impedance between the pulses; this arrangement prevented electrode polarization during the stimulation train. The current intensity and temporal parameters of the stimulation were continuously monitored on a Tektronics 502A oscilloscope that read differentially across a 1K Ω precision resistor in series with the electrode.

All of the temporal parameters of the ESB were controlled by automated, solid state, logic modules (Grason-Stadler Corporation, 1200 series). Each set of ESB parameters was tested for 140 sec, at the end of which time the total duration of the stimulation, the number of stimulations and the P were printed out by a print-out counter. The ON and OFF latencies were calculated according to the following formulas.

$$\text{OFF Latency (av. ESB on time)} = \frac{\text{Total ESB Duration}}{\text{number of stimulations}}$$

$$\text{ON Latency (av. ESB OFF time)} = \frac{\text{Total test duration (140 sec)} - \text{Total ESB Duration}}{\text{number of non-stimulations}}$$

[6]

[6] Because each 140 sec period started with the stimulation on, the number of non-stimulations was assumed to be equal to number of stimulations-1. When this resulted in impossible latencies (>20 sec), ON latency was recalculated using number of stimulations, and the best estimate was used.

Following the data printout, the P of the ESB was automatically increased by .1 log unit (about 25%) and a new 140 second test begun immediately. Values of P were chosen such that at least the first two P's tested resulted in vigorous ON and OFF responses. The ascending series of P's would continue until the animal had emitted neither response for at least two successive 140 sec test periods. Before the next series of P's was tested, the animals were given two 140 sec test periods with no current. In this manner, curves relating P to ON Latency ($P\text{-}L_{ON}$) and P to OFF Latency ($P\text{-}L_{OFF}$) were generated simultaneously (the curves generated by joining the data points usually resembled cumulative normal curves).

By varying a second parameter of the ESB (eg. intensity) families of $P\text{-}L_{ON}$ and $P\text{-}L_{OFF}$ curves were generated. The Intensity was held constant while P varied to produce one $P\text{-}L_{ON}$ and one $P\text{-}L_{OFF}$ and then the intensity would be changed to produce a second set of P-L curves. This allowed the determination of the P required to produce a given response latency for any given intensity (see Figure 2a, b), i.e. the P required to reach a constant output for the intensity. This P is the constant output period (COP) and this basic procedure was used for determining both P-I trade-off functions (eg. Figure 2c) and the functions relating T_E to the C-T interval. (eg. Fig. 3).

The COP's for pulse-pair conditions were all determined at a single intensity for each animal. To determine the COP for a particular C-T interval, the C-T interval was set and then the regular P series was run such that there were at least two 140 sec test periods with vigorous ON and OFF responding at the beginning and at least two 140 sec periods with no responding at the end. The COP was always the

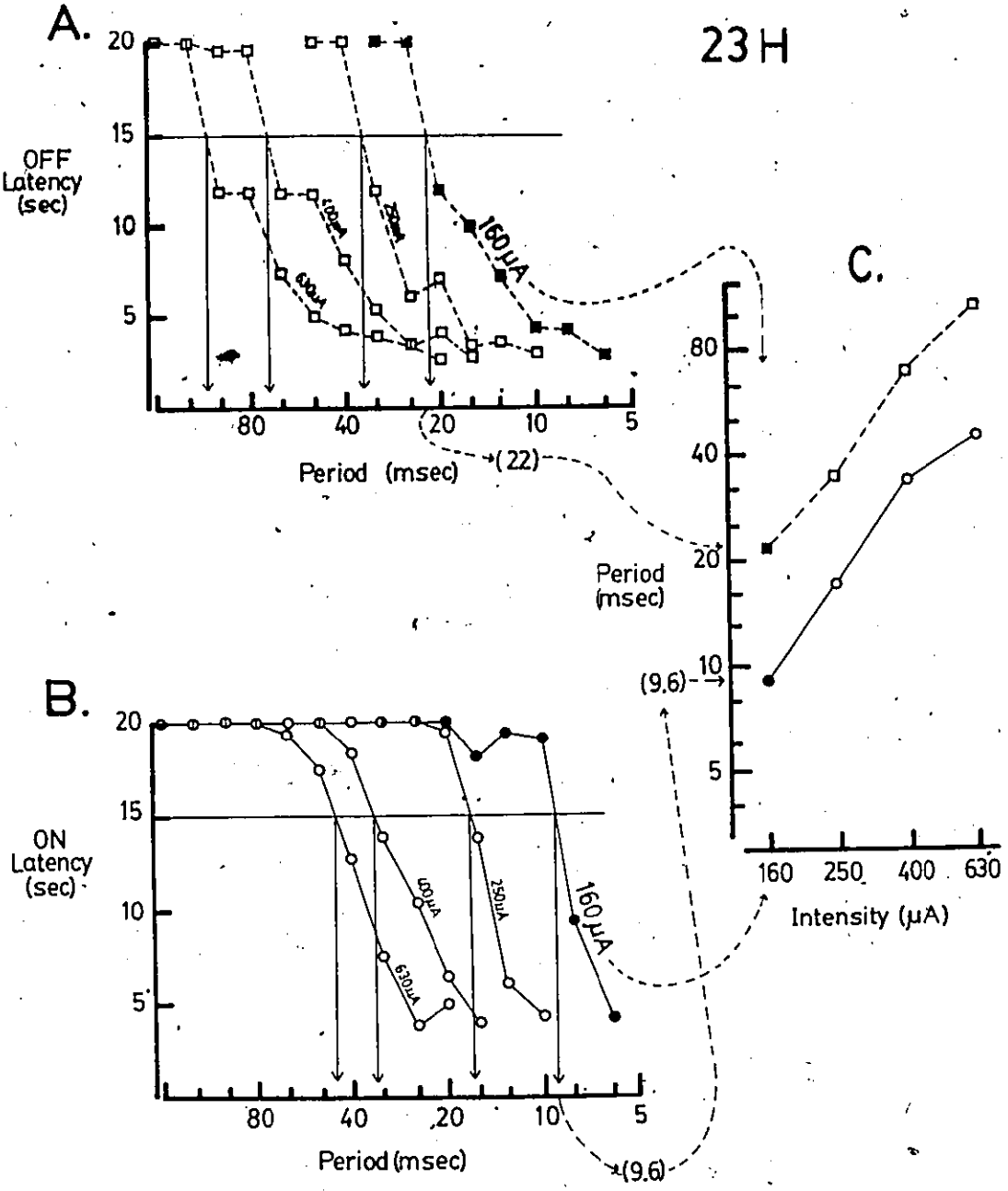
Figure 2. The derivation of a P-I trade-off function. Data from animal 23H obtained during a single day's testing.

A. Period versus OFF Latency. Each square within this set of axes represents the mean latency (ordinate) of the OFF responses made during 140 sec test of a particular intensity (I) and period (P, abscissa, log axis). The dashed lines which join the mean latencies describe the Period-Latency (P-L.) function. The numbers along each P-L. function show the (I) at which the function was obtained. The thin straight line drawn parallel to the abscissa from the ordinate value of 15 sec shows the arbitrary constant-behavioural output from which the trade-off function was derived. The P at which this constant output occured (COP) was found by dropping a vertical line from the intersection of the P-L curves and the constant output latency to the absicissa. The mean latency values for 160 μA are solid in order to make them distinctive. For clarity, only the P-L curves from every second I tested are shown on this graph.

B. Period versus ON Latency. Each circle within this set of axes represents the mean latency of the ON responses made during the same 140 sec tests as in A. The ordinate, abscissa, constant output, P-L curves and COP derivations are the same as in A. Note that the COP's are different and that the mean latencies for 160 μA are again solid for distinctiveness.

Figure 2 (con't)

C. Period vs Intensity (P-I) Trade-off Functions. The squares and straight dashed lines show the trade-off between P and I derived from the P-L curves of A (the OFF response). The circles and straight solid lines show the trade-off between P and I derived from the P-L curves of B (the ON response). The curved dashed lines show the translational process. The I of each P-L curve is plotted on the abscissa (log scale) and the COP of each P-L curve is plotted on the ordinate (log scale). The points of each curve derived from the P-L curve at 160 μA are solid for distinctiveness.


A.
23 H
OFF Latency (sec)
20
15
10
5
80
40
20
10
5
Period (msec)
(22)
160 µA
C.
Period (msec)
80
40
20
10
5
(9.6)
160
250
400
630
Intensity (µA)
B.
ON Latency (sec)
20
15
10
5
80
40
20
10
5
Period (msec)
(9.6)
400µA
250µA
160 µA
630µA

point at which the L-P curve crossed the 15 sec latency (see Figure 3). Families of five to seven curves were run in sessions shorter than 4.5 hours. Pilot studies revealed that testing animals longer than 4.5 hours resulted in unstable behaviour.

Three types of curve families were collected: stabilization curves, P-I trade-offs and double pulse curves. Stabilization curves were run to train the animals. This training consisted of testing the animal repeatedly on the same P series at a single I for 4.5 hours and was continued on alternate days until the range of the COP's over the seven curves of both behaviours were less than .2 log units. The stabalization intensities were chosen so that both behaviours were vigorous at P=10 msec and neither ON nor OFF responding occured beyond P=50 msec. Animals were discarded if an intensity that produced these behaviours within these P limits could not be found.

Screening, Training and Testing

After a week of post-surgical recovery, the animals were connected up to the stimulator and placed in the test chamber. The animals were shaped to press the ON-lever with brief (half-second) trains of ESB with P around 10 msec. The I was increased from 200 μAmps until a single train would lead to either vigorous exploratory behaviour or aversive-like effects (vocalizing, defacating, etc.). When the animals had learned to press the ON lever for .5 sec trains of ESB, they were allowed to continue for at least an hour and were sometimes tested for several days on this one behaviour. Once the animals were adept at turning the ESB on, the train length was increased to a maximum of twenty seconds and the animals were shaped to turn it off

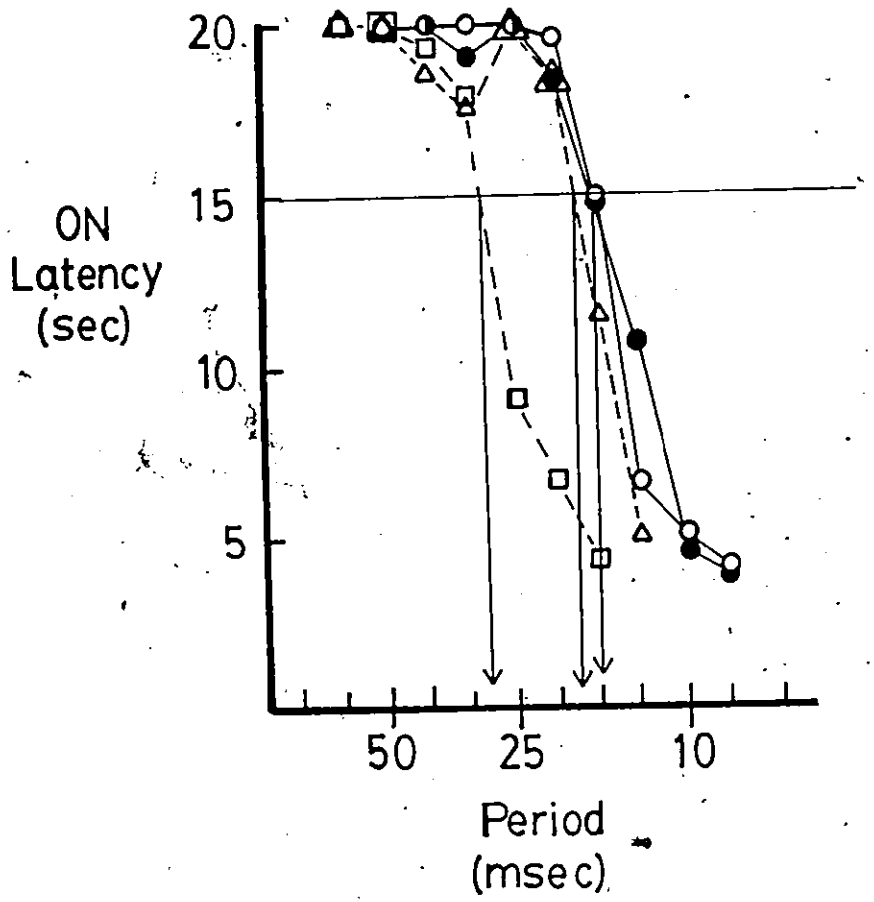
Figure 3. Derivations of the constant output periods for single pulse (COP_{SP}) and double pulse (COP_{DP}) conditions. For graphic clarity, only certain DP conditions are shown. All data for both sets of curves were generated during a single 4.5 hour test session of animal 23H. The tables show the $COP_{SP's}$ and the $COP_{DP's}$ derived from the data.

a. Curves relating ESB period to the animal's latency to turn the stimulation on (ON Latency). These are $P\text{-}L_{ON}$ curves.

b. Curves relating ESB period to the animal's latency to turn the stimulation off (OFF Latency). These are $P\text{-}L_{OFF}$ curves.

a.

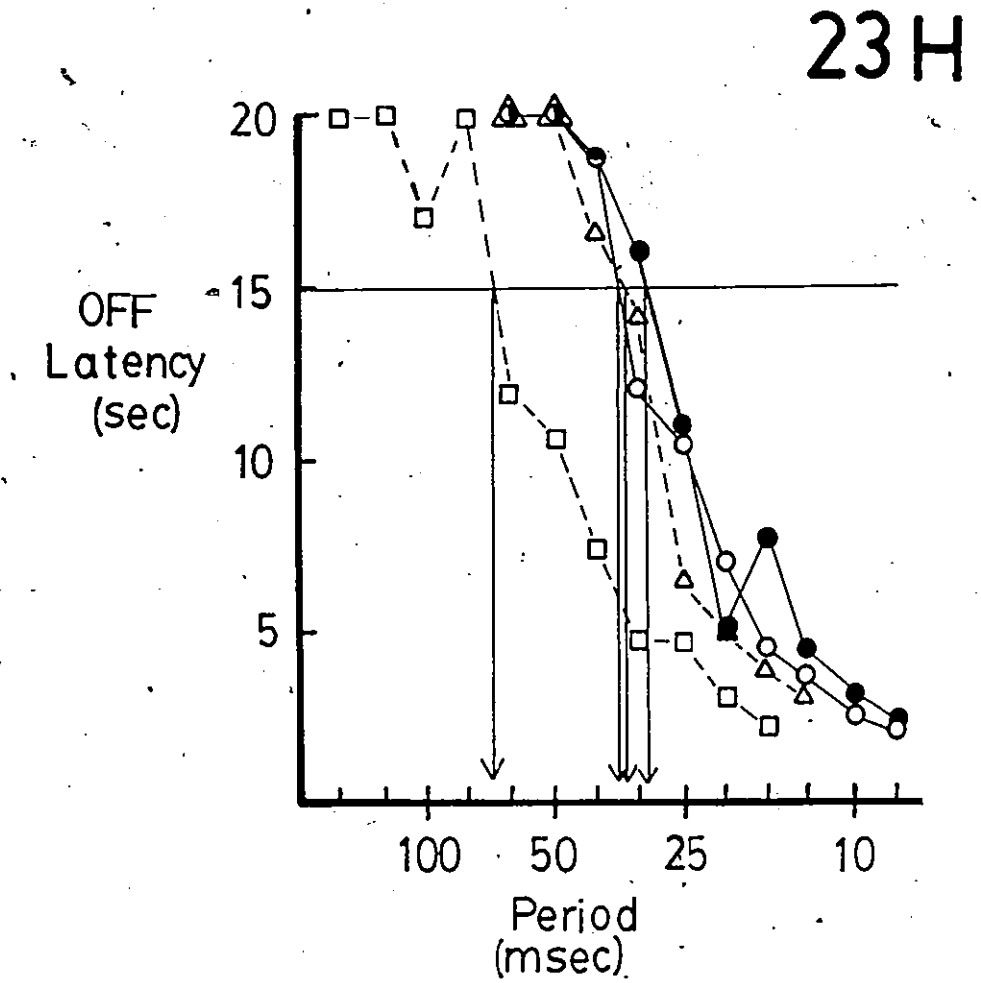
23H



	C-T Interval	COP_{DP}
△--△	0.6	18
(not shown)	0.2	18
	1.0	24
□---□	5.0	29

	single pulses	COP_{SP}
●—●	first	16
○—○	second	16
	mean	16

b.



	C-T Interval	COP_{DP}
△--△	0.6	35
(not shown)	0.2	42
	1.0	55
□--□	5.0	69

	single pulses	COP_{SP}
●—●	first	30
○—○	second	36
	mean	33

by successive approximations. The ESB parameters were set high enough to produce reliable escape movements but low enough to be non-disruptive.

Animals that did not learn both responses over three training sessions were not used. Electrodes that produced motor effects, convulsions, or interfering behaviour (eg. tail-carrying) were not used. If any of these extraneous effects of the ESB appeared during the subsequent testing of the animals, testing was immediately discontinued and the animals' data were not used in the final analysis. The data from the only animal to lose its electrode during testing were also not used.

All animals progressed through the various phases of the study at their own rate, completely independant of all other animals. After each animal had stabalized its COP's, a period-intensity (P-I) trade-off was run. On the basis of the P-L curves collected during these runs, the I to test for RP estimates was chosen. The I for each animal was the lowest I that gave orderly P-L curves for both behaviours and latencies less than 10 sec at P=10 msec and latencies greater than 15 sec when P=50 msec. The animal was stabilized with this I until all COP's of each behaviour had a range of less than .1 log units and then testing with pulse pairs was begun.

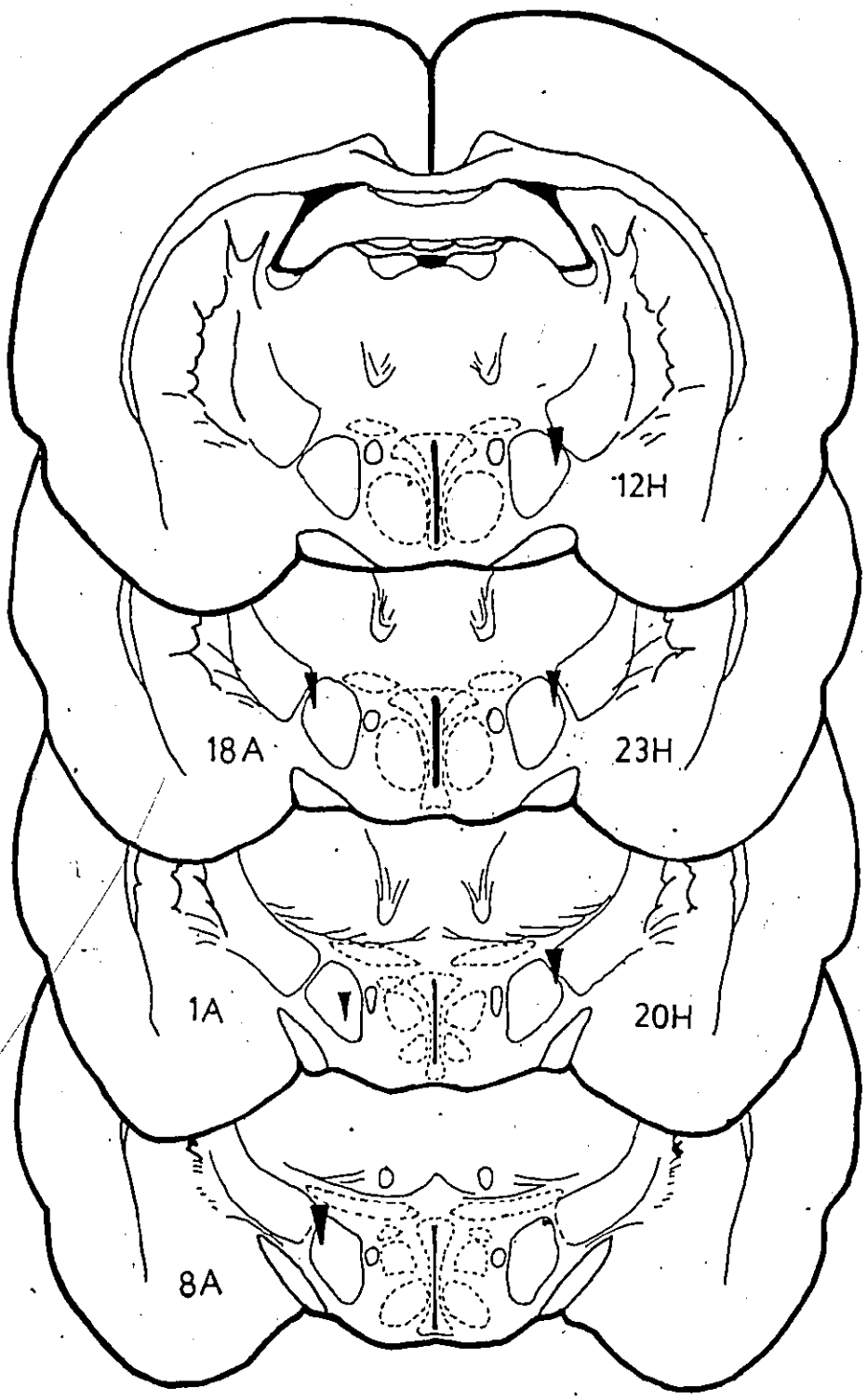
Each 4.5 hour test session began and ended with P-L curves using single pulses (SP). The 4 or 5 other P-L curves that were run between these two SP curves were all double pulse (DP) curves. The C-T interval was set during the two 140 sec no-current test periods and then maintained through the P series. The C-T intervals (in msec) tested were 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 2.0, and 5.0. All nine C-T intervals were tested during two successive test days.

The order of presentation was counterbalanced three different ways over the six repeated testings of the series.

Test sessions in all phases were run on alternate days at roughly the same time of the day for any particular rat. Most animals were tested in the evening. For all animals, the twelve DP test sessions were run within a 24-day period. Upon completion of all the RP tests, the animals were again tested for their P-I trade-off functions. Most of the rats then were used in various pilot experiments before being sacrificed for histology. Six rats completed the full test series[7]: 1A, 8A, 12H, 18A, 20H, and 23H. The histologically determined electrode locations are shown in Figure 4.

[7] The letters refer to the electrode location, A refers to electrodes aimed at the left LH and H refers to electrodes aimed at the right LH.

Figure 4. The location of the stimulating electrodes of the six animals who completed this study. They are located on tracings taken from successive plates of Konning and Klippel's (1963) atlas. The plate numbers appear on the right side. The number-letter combination represent the animal number and the side of the brain respectively, A for left, H for right. The ▮ represents the length and width of the exposed tip of the electrode, drawn to scale.


29
12H
30
18A
23H
31
1A
20H
32
8A

Results and Discussion

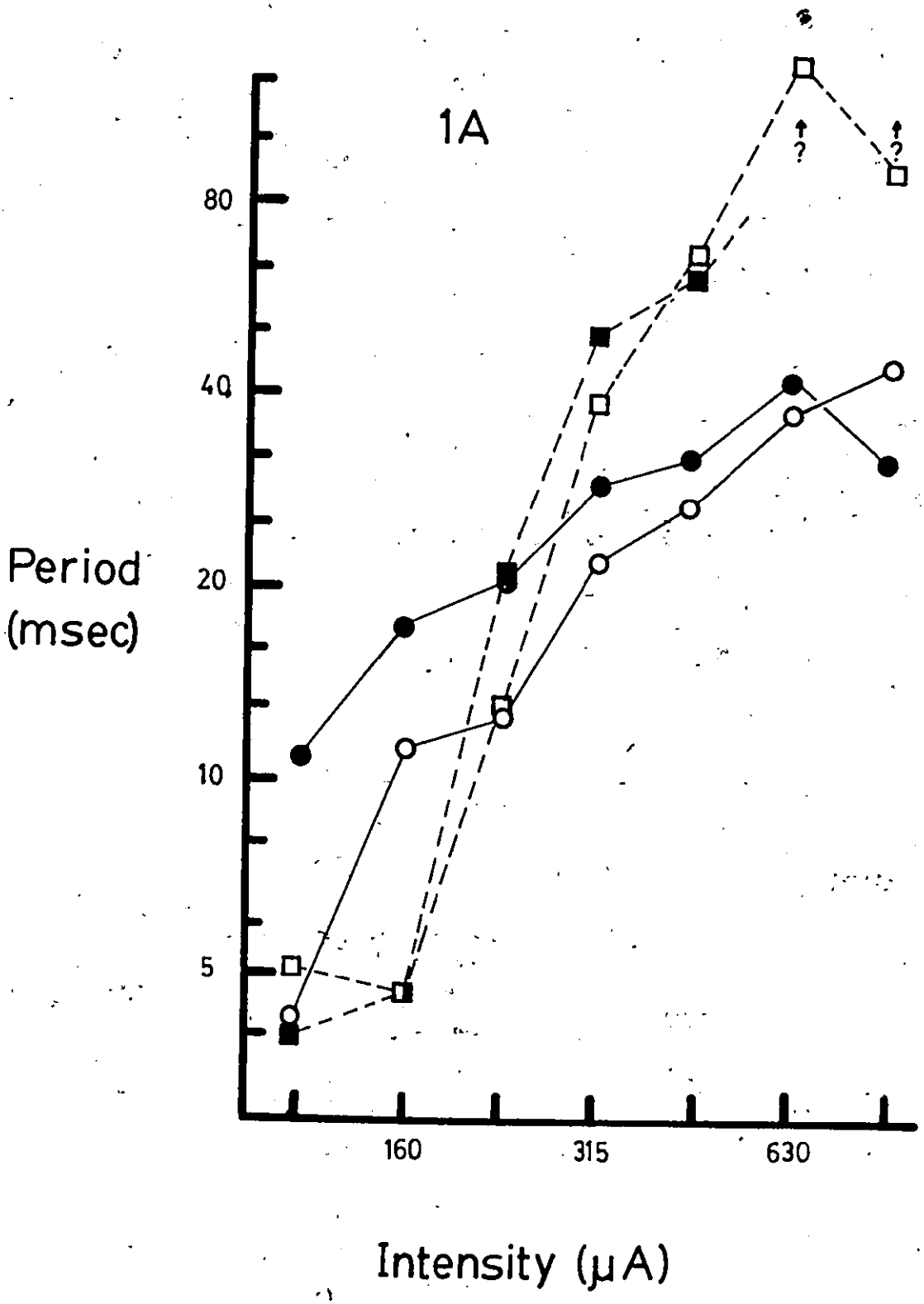
I. Period-Intensity (P-I) Trade-offs.

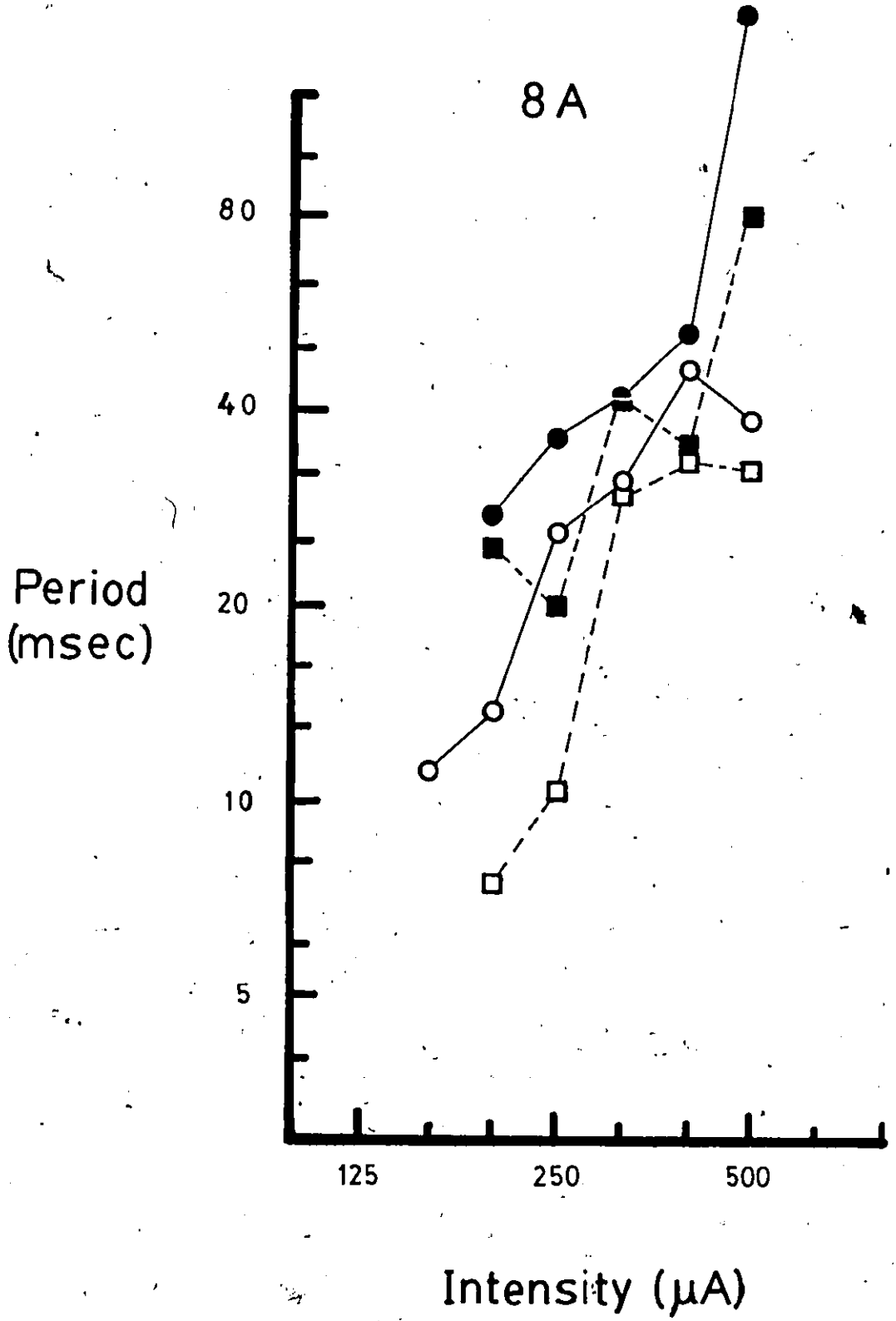
The pattern of behaviour consistantly observed was an increase in the latencies of the ON and OFF responses as P increased while I was held constant. This pattern can be seen if the representative Period-Latency (P-L) curves of Figure 2 are read from right to left, the way they were run. When the I was changed, the whole P-L curve shifted such that I increases led to higher COP's, and I decreases led to lower COP's (see Fig. 2). Trade-off curves were derived by plotting I against COP. The curves for both trade-off determinations of both behaviours are presented for the six animals in Figure 5.[8]

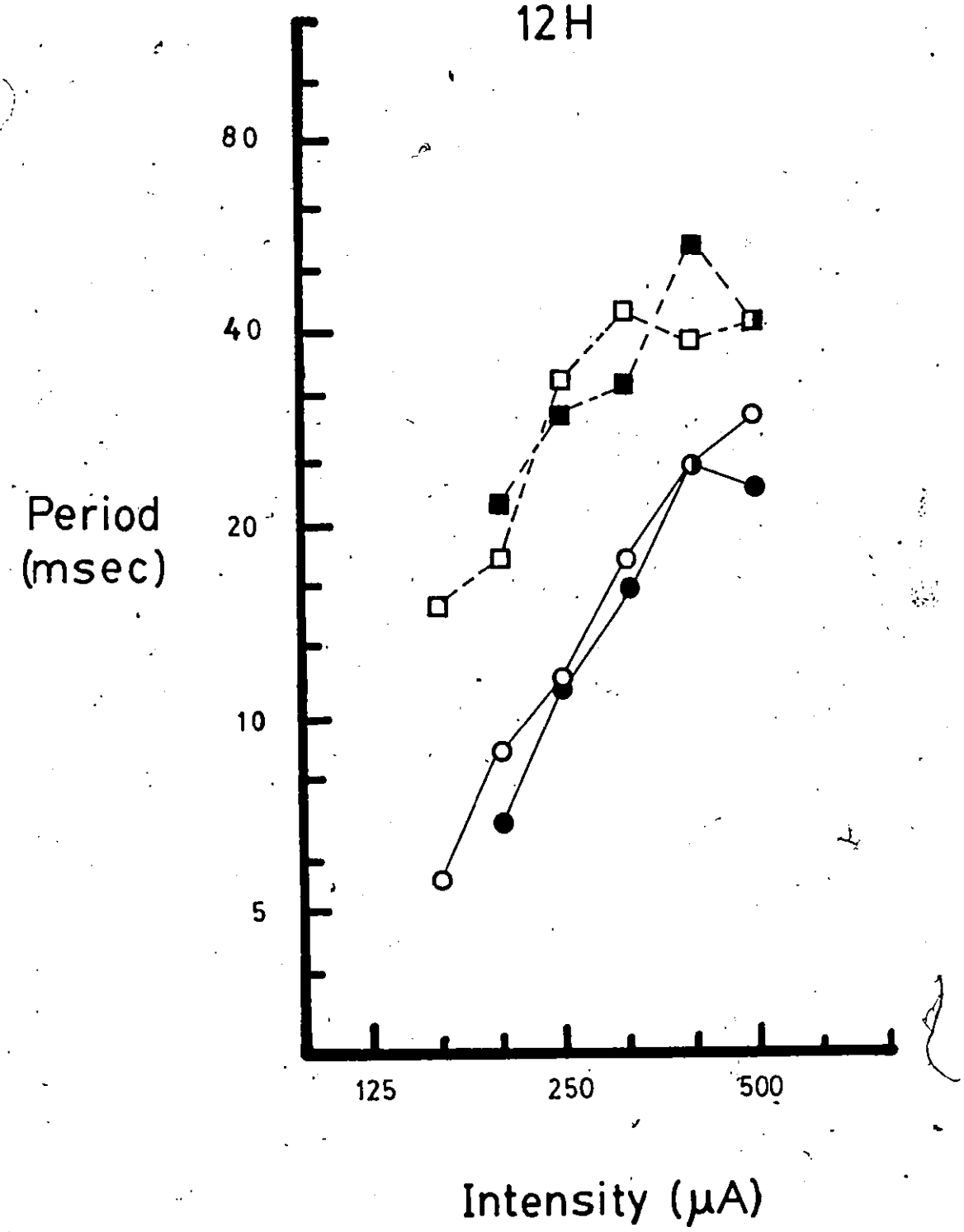
These curves generally show a systematic trade-off between P and I within certain ranges. They indicate an orderly relationship between the intensity of the pulses and how closely they must be packed in time in order to produce a particular level of behaviour. The curves are not superimposable for animals or behaviours or even for the two determinations in each animal. Visual examination of the curves indicates that the differences in the curves between the two behaviours were often greater than the differences between the two determinations of the trade-offs for a given behaviour. The differences between the behaviours were clear for some animals (1A, 12H, 23H), but not for others (8A, 18H, 20H).

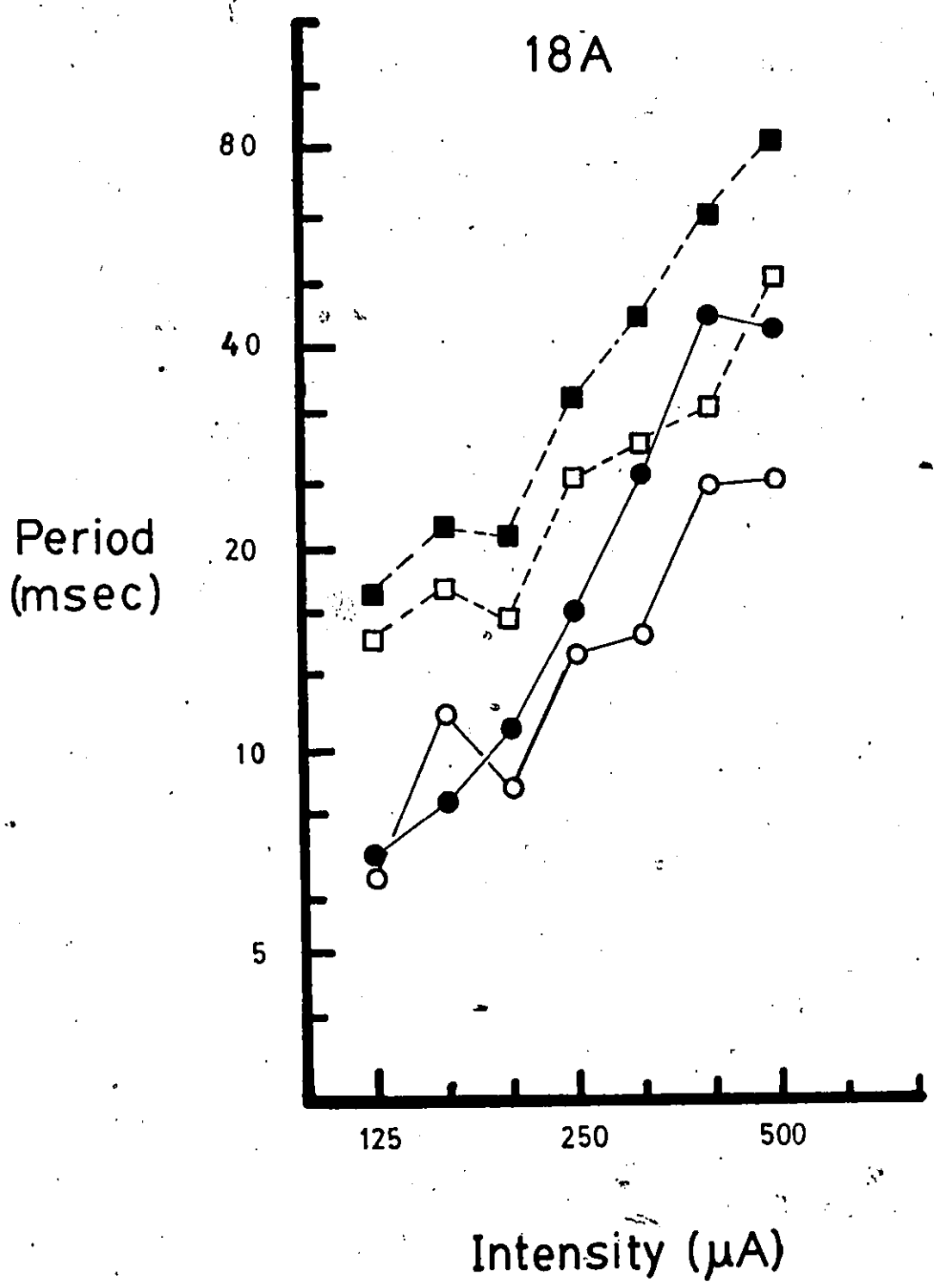
[8] Although the P-I trade-offs were determined for at least two criteria (constant-outputs), the level chosen did not systematically affect the shape of the P-I curves and so for simplicity, only one set is presented here.

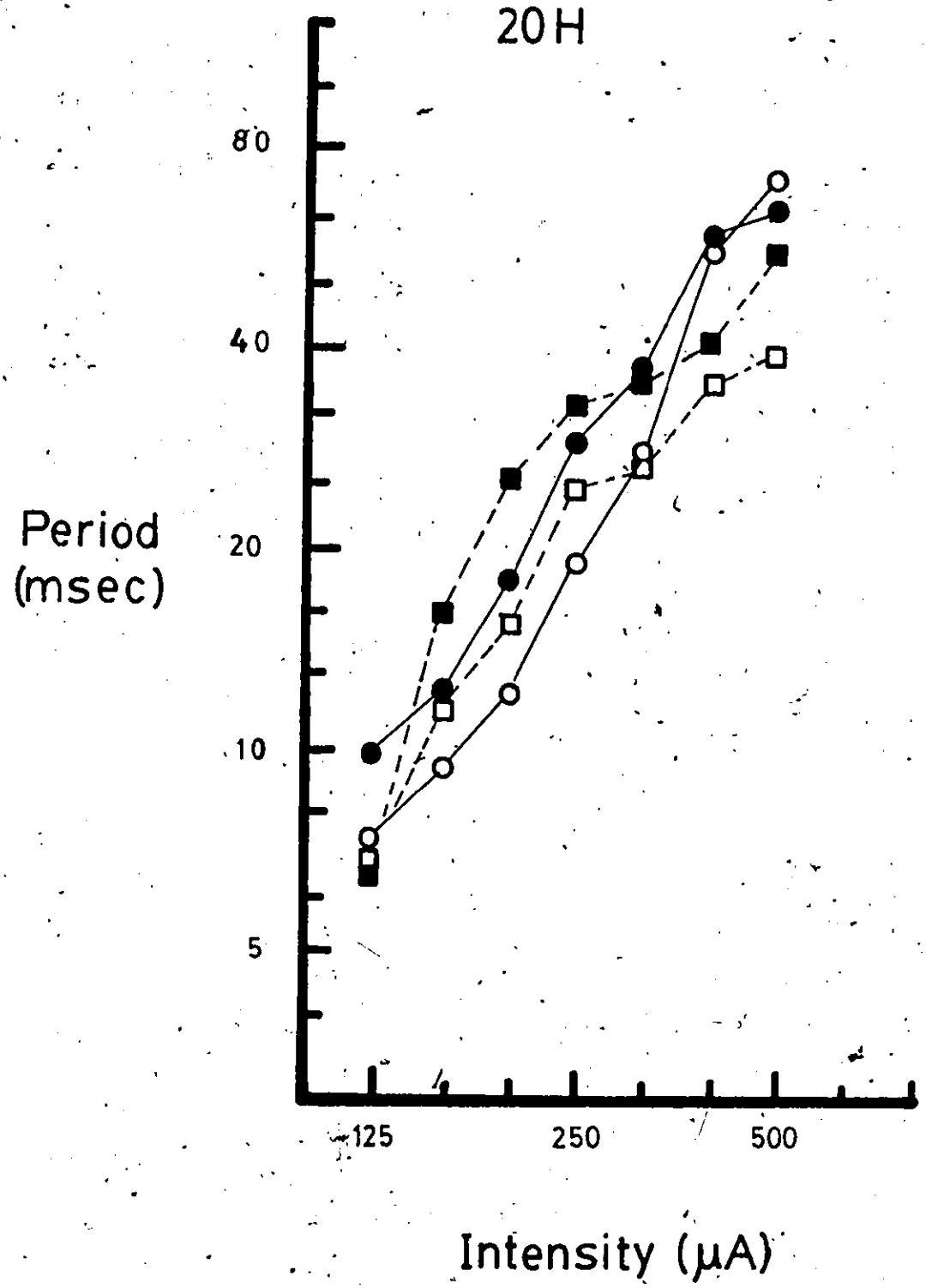
Figure 5. Graphs of the Period-Intensity (P-I) trade-offs for the six animals, plotted on log-log axes. The numbers in the upper left hand corner represent the animals and the location of their electrodes (A = left LH, H = right LH). The solid lines represent the trade-offs of the ON response, the dashed lines represent the OFF response. The open symbols represent the first determination, the closed symbols represent the second determination. The question marks on the trade-off functions for animal 1A represent the minimum COP for those two high I's. Initially, the stimulation programming devices were limited to P's less than 100 msec.

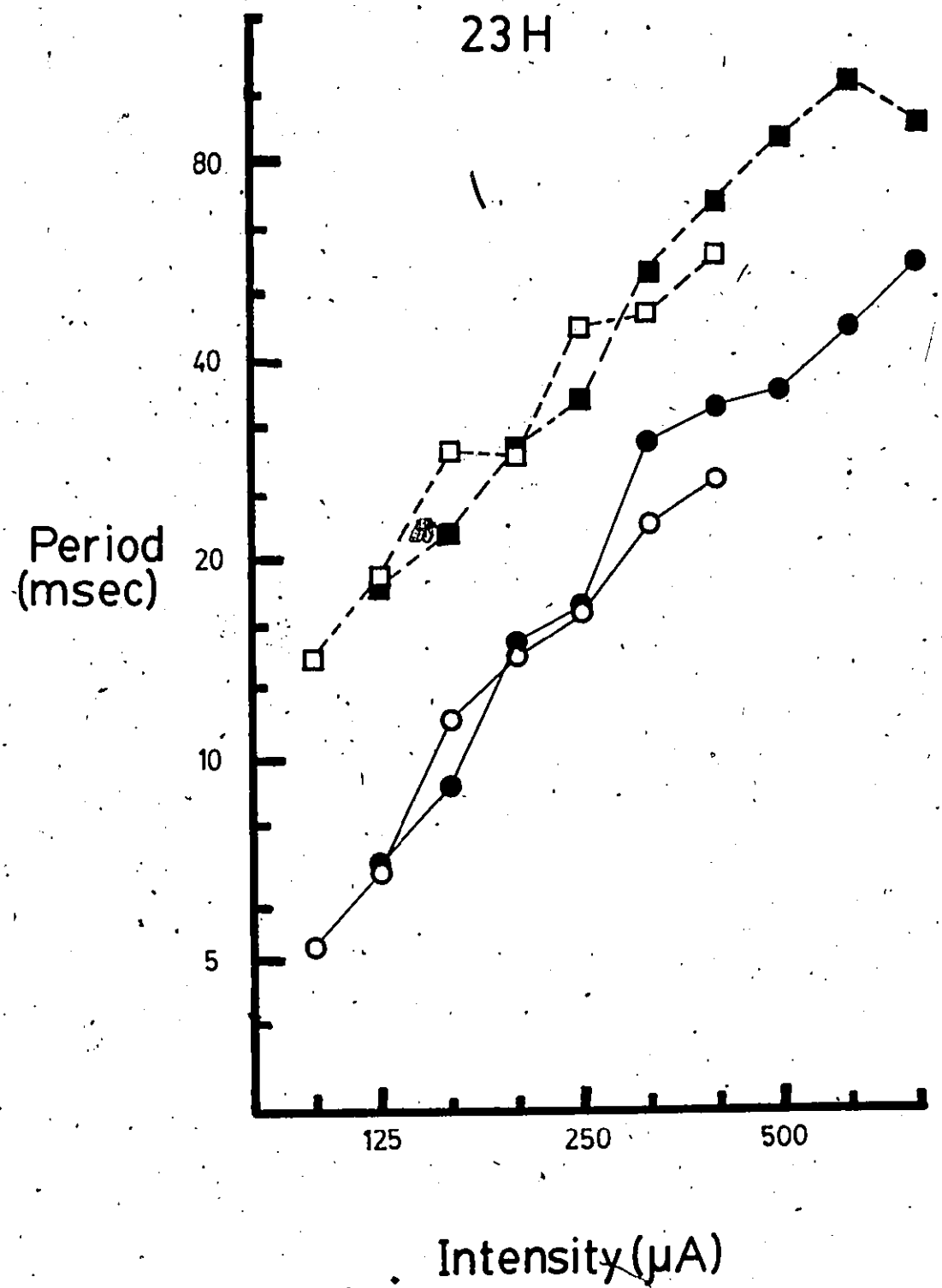

1A
80
40
20
10
5
Period
(msec)
160
315
630
Intensity (μA)


8A
80
40
20
10
5
Period
(msec)
125
250
500
Intensity (μA)


12H
80
40
20
10
5
Period
(msec)
125
250
500
Intensity (μA)


18A
80
40
20
10
5
Period
(msec)
125
250
500
Intensity (μA)


20H
80
40
20
10
5
Period
(msec)
125
250
500
Intensity (μA)


23H
80
40
20
10
5
Period
(msec)
125
250
500
Intensity (μA)

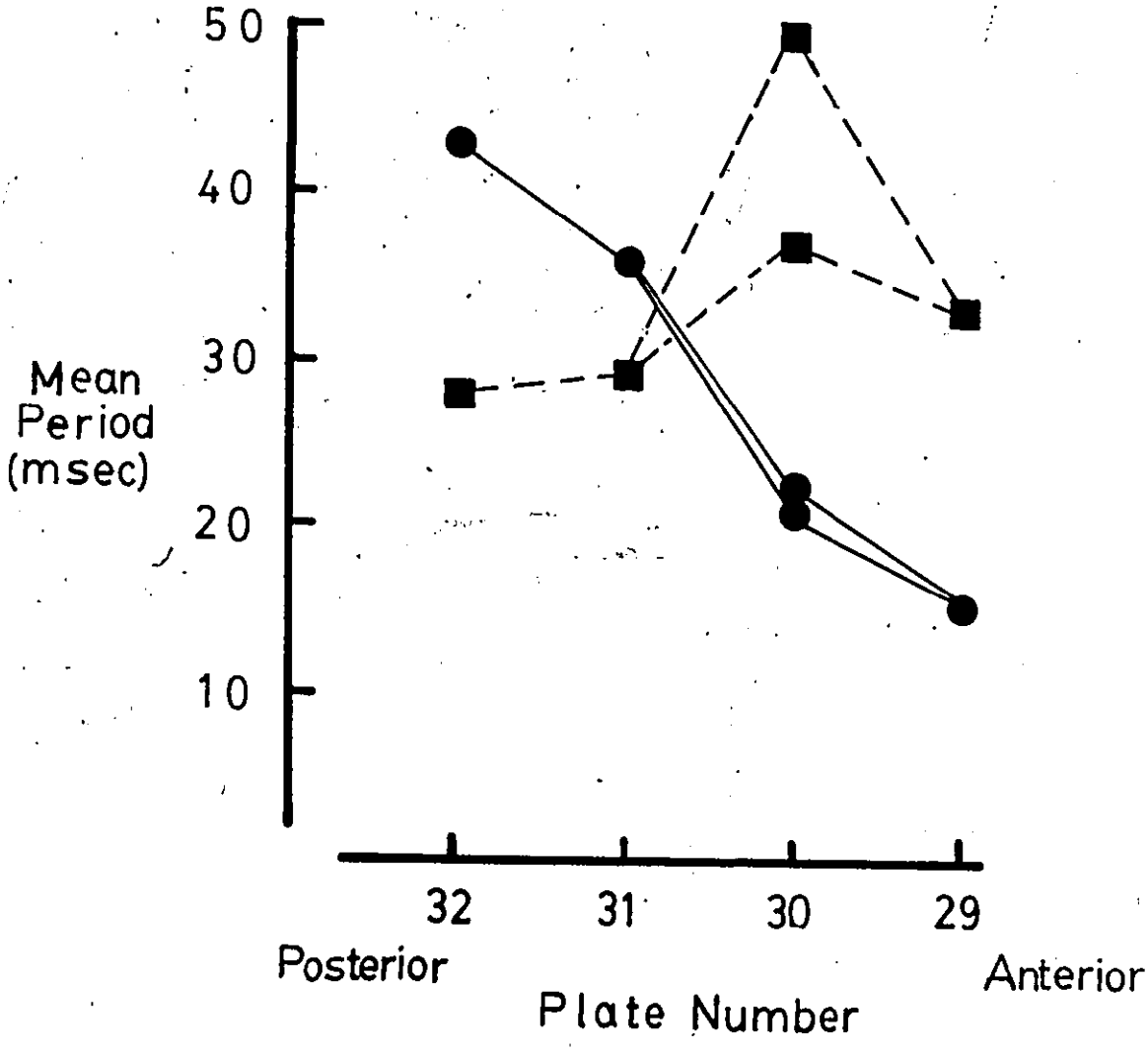
Following the compilation of the trade-off and histological data, an interesting but unexpected relationship between the P-I curves and electrode location emerged. It appeared from histology that all electrodes but one (1A) had approximately the same spatial relationship with the internal capsule and the LH. The major distinction between the electrodes was in the A-P direction. It became apparent that the P range of the P-I trade-off curves for ON responding was related to the A-P plane but that the P range of the curves for OFF responding were not. In order to illustrate this relationship, the mean P across I's between 160 μA and 500 μA were calculated for each animal for each behaviour. The results of this averaging procedure are shown in Figure 6. These curves seem to show systematic changes in average COP for the ON response but not for the OFF response. In order to interpret this finding as well as the P-I trade-off curves themselves, it is necessary to examine some theoretical explanations of why P and I should trade-off at all.

Gallistel and others (Edmonds, Stellar and Gallistel, 1974; Gallistel, 1974, 1976; Shizgal, 1975; Shizgal and Matthews, 1977) have developed physical and mathematical models to explain why different parameters of the ESB have been found to trade-off against one another in the manner shown by their data. Although the P-I trade-off data presented here will be shown to be consistant with their data and their model, the full development of the model is beyond the scope of this thesis and so only an intuitive explanation will be given here.

The Counter Model

The simplest physical model of ESB effects treats an intracranial,

Figure 6. This graph shows the plot of mean Period (P) against the plate number of Koning and Klippel's atlas upon which the electrode locations were best superimposed. The mean P was determined across current intensities ranging from 160 μA to 500 μA. The solid lines connect the mean P's for ON responding and the dashed lines connect the mean P's for OFF responding. Note that each behaviour is represented by two points at Plate 30 because there were two electrodes located in this A-P plane.


50
40
30
20
10
Mean
Period
(msec)
32
31
30
29
Posterior
Anterior
Plate Number

monopolar, cathodal electrode as if it were a point current sink in the middle of a volume conductor. According to this simplification, the current density is related to the inverse square of the distance from the electrode. Because the depolarization effect of the ESB on any particular neuron is related to the local current density, the depolarization effect is also related to the inverse square of the distance from the electrode (Ranck, 1975). Although the orientation, threshold and membrane properties of any particular neuron, as well as the resistivity of the intervening tissue, determine whether or not that particular neuron will fire to a pulse of a given intensity and pulse width, empirical evidence suggests that for any given direction, the relationship between current intensity and distance to the electrode seems to hold fairly well (Ranck, 1975; Matthews, 1975).

If the electrode were in the middle of the large homogeneously distributed population of neurons which all had the same threshold, and if the resistivity of the tissue was homogeneous, it would be fairly simple to predict the consequences of a stimulating pulse, when certain neurophysiological concepts are used. A pulse of a given intensity would depolarize a neuron by an amount related to the distance of that neuron from the electrode tip. If the intensity was high enough, neurons near the electrode would be depolarized beyond their thresholds and action potentials (AP) would result. Further away, the current intensity would be strong enough only to depolarize the neurons just to threshold. While these neurons would be fired, those beyond them would register only subthreshold depolarizations that result in local (non-propagated) potentials (LP). The distance to

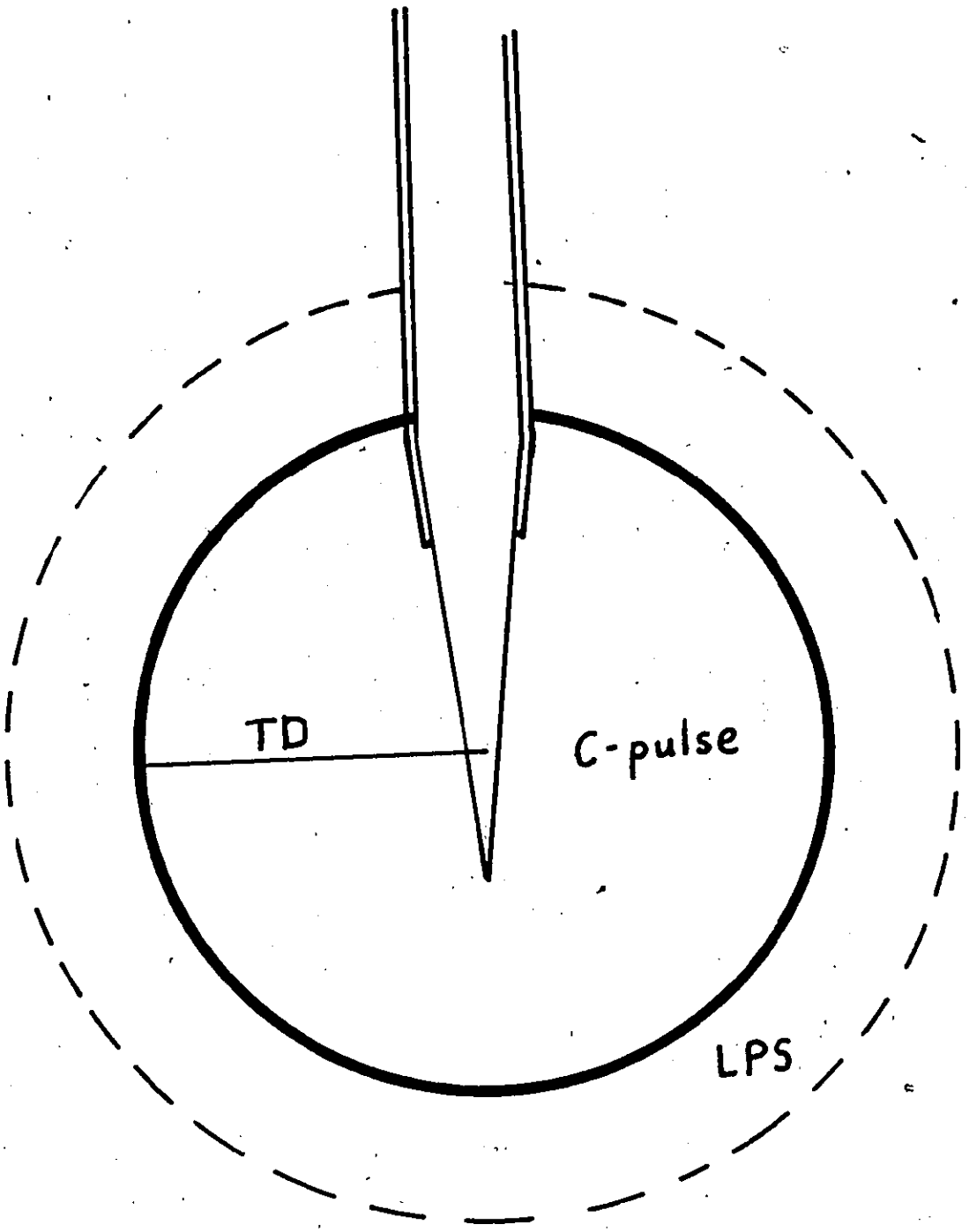
which the stimulation depolarizes neurons to threshold will be considered to be the threshold distance (TD).

The concept of threshold distance can be applied to the more complex (and probably more realistic) case of an electrode tip in the middle of a mixed group of neurons with various current thresholds and where the resistivity of the tissue around the tip is not necessarily homogeneous. The threshold distance will vary with the neuron's threshold and the intensity contours of the now more complex stimulation field, but any neuron can be characterized according to whether it is nearer to the electrode than the TD or farther away. All neurons closer than the TD will be fired by the stimulating pulses and those just beyond the TD will have LP's generated. Even though current may not spread equally in all directions due to local areas of low or high resistance (eg. blood vessels or mylinated fiber tracts), the spatial relationship between any neuron and the electrode tip can be described in terms of the TD for that neuron and that direction. The spread of current around an electrode tip can, therefore, be modeled as a sphere with a radius equal to the TD without introducing any very serious errors. The cross-section of this sphere is represented by Figure 7 in which different regions are labelled on the basis of the magnitude of the depolarizing effect of the stimulation pulse.

The number of neurons fired by each pulse will depend upon the number enclosed by the sphere defined by the TD.[9] The size of the

[9] The number of axons of passage enclosed by the sphere will be related to the cross-section area of the sphere normal to their direction. The number of cell bodies on the other hand will be related to the volume of the sphere.

Figure 7. A schematic diagram showing the model of effects of a stimulating pulse. The diagram represents the cross section of the sphere of current spread. All areas are defined in terms of the effect of the stimulating pulse on the surrounding neurons. The pulse fires neurons out to the threshold distance (TD), represented by the solid line. The dashed line represents the distance to which neurons have their membrane potentials depolarized to at least 1/2 threshold. The region between the solid and dashed lines is the area in which relatively large local potentials (LPS) are generated by the pulse.


TD
C-pulse
LPS

sphere will be determined by the stimulation intensity and pulse width, the tissue resistivity and the threshold and membrane characteristics of the neurons in question. When these other factors are maintained at a constant level, the number of neurons enclosed will be related to the current intensity. The other major factor determining the number of neurons fired by a stimulating pulse is the spatial distribution of the neurons themselves.

In the present context, only select populations of neurons around the electrode tip are of interest. The stimulation could well excite many neurons whose activity does not produce any effect upon the behaviour(s) being examined. There are some populations, however, whose activity does effect the behaviour(s) being examined. These will be called the behaviourally-relevant neurons.[10] Henceforth all discussion will be limited to these neurons.

The spatial relationship between these neurons and the electrode tip will vary with electrode location. The density of the neurons near the electrode tip depends upon the anatomy of neural structure and the proximity of the electrode relative to the structure. When the electrode is reasonably close to the neurons (as shown by the ability of the ESB to affect the behaviour), increases in I will increase the number of

[10] Of course, the neurons upon which the directly stimulated neurons synapse are no less relevant to the behaviour. In order to distinguish the directly excited neurons from those excited monosynaptically, these latter neurons will always be refered to by the term "follower neurons". The model presented here does not attempt to explain the many neural events that must intervene between the activity in these follower neurons and the actual behaviour.

neurons stimulated, within certain limits. Around any electrode there is a region of scar tissue within which there are no neurons whatsoever. Very low I's will, therefore, be unable to fire any neurons at all. At the other end of the scale, increasing I beyond a certain point may not change the number of relevant neurons fired, for it is only reasonable to assume that the population(s) is finite and bounded in space.[11] Between these limits the relationship between I and the number of neurons should be as uniform as the distributions of the neurons themselves.

The formulation of the effects of I on the relevant neurons is an intregal part of the counter model but because no single pulse, no matter how long or intense, has been demonstrated to be capable of acting as a sufficient reinforcer for operant behaviour, the counter model was developed by Gallistel and others to explain how the effects of successive pulses summate over time. On the basis of trade-offs of P and I, I and the number of pulses (N), and N-I trade-offs derived from runway experiments using ESB as the operant reinforcer, Gallistel proposed that the "follower neurons" mediating the positive reinforcement effect acted as a perfect integrator for a brief period following ESB onset and then acted as a leaky integrator after that. The output

[11] In fact, if the I's are large enough, the current density very close to the electrode may be high enough to block AP in neurons close to the electrode. Although the effect of this "anodal surround" (Ranck, 1975) may be very slight at most I's used in this study (Yeomans, 1977), the effect can not be ruled out entirely at high intensities.

of this integrator was said to reflect the number of action potentials received during these periods of integration. The number of action potentials that function as the quantity to be integrated was proposed to be the product of the number of input channels (neurons) and the number of action potentials per channel. When the ESB duration is constant, the number of action potentials per channel is determined by the frequency (F) of the ESB. This assumes that the pulses are short enough to cause only one firing per channel each and that they are spaced sufficiently in time to allow the recovery of membrane excitability between pulses.

This model reveals the limits of P. The lower limit of P is determined by the post-stimulation excitability cycle of the relevant neurons. Each neuron must have recoverd from the effects of one pulse before it can react in the same manner to the next pulse.[12] If P is very large, the effects of each AP might decay between pulses to such an extent that there is very little summation between pulses. In addition to this limit on P, there must be a limit to the durations that can be integrated. The output of the integrator cannot reflect the sum total of all past inputs but must be restricted to relatively recent events. Consequently it has been proposed that the integrater "views" its input through a time-window, (the period of integration).

As mentioned earlier, Shizgal, (1975) and Shizgal and Matthews, (1977) found that increasing the duration of bursts of stimulation beyond a second or so did not change the ON response while increases in the

[12] The second half of this thesis deals with this factor in detail.

duration of the stimulation did affect the OFF response. It appeared that there was a difference in the "time-windows" through which the systems responsible for the two responses integrated the ESB generated activity. This finding is very important for the application of the counter model to the two-lever shuttle paradigm used here.

The latency of the ON response was found to be related to the parameters of the ESB. The duration of the ESB, however, was determined by the OFF latency. The OFF latency, therefore, was one of the factors that determined the number of stimulating pulses and so the number of AP's received by the integrator. However, because Shizgal showed that the ON latency depended only upon the first second or two of stimulation (presumably because of the "time-window" of the ON integrator) and because the OFF latencies were never as short as two seconds, it can be safely assumed that the ON integrator received stimulation throughout its whole "time-window" during each ESB presentation. The counter model proposes that the latency of any ON response is related to the ON integrator's sum that resulted from the immediately preceeding ESB. If the "time-window" of the ON integrator was the same throughout the test session, then the sum in the integrator should have been related only to the P and I of the ESB.

The sum of the OFF integrator on the other hand, was expected to reflect the summation of the activity caused by most, if not all, of the ESB duration. The long period of integration in the OFF system indicated that it integrated the ESB induced activity until a sum was reached that was sufficiently high to warrent an OFF response. Once this level had been reached, a certain amount of time was required to

execute the response. Shizgal (1975) pointed out that if this execution time was reasonably constant and relatively short in relation to the total OFF latency, then the OFF latency would be a reasonable estimate of the time required to achieve the integrator sum sufficiently higher to warrent the OFF response.

The use of the 15 sec latency as the criterion value in determining effects of parametric ESB manipulations contributed in two ways to the approximation of the period of integration by the OFF latency. When the ESB parameters were adjusted to produce 15 second OFF latencies, each animal's behaviour was very orderly. They would usually stand near the OFF bar for several seconds, and then reach out and press it. Behavioural observations indicated that during these trials, the actions necessary for the response required a reasonably invariant and relatively short length of time (one or two seconds). Because all the trade-offs were taken at the same OFF latency (15 sec), the duration of the ESB required to drive the OFF integrator to its required sum (15 sec - execution time) was fixed. It is, therefore, expected that the trade-offs of P and I in the present experiment should reflect the ability of the integrator in the OFF system to trade-off the neural effects of P and I.

In order to examine how P and I affect the output of the integrator, it is not necessary to be able to specify exactly how the output of the integrator is related to the behavioural output of the animal. As long as the relationship is monotonic and stable, it is a reasonable assumption that a particular behavioural output reflects a particular sum in the integrator. It is the nature of the trade-off functions to determine how much one variable (eg P) must be changed in

order to obtain the same behavioural output (eg. Latency = 15 sec) after another variable has been changed (eg. I). Stated differently, the trade-off procedure determines how various levels of two variables (eg. P and I) can be paired to achieve a constant behavioural output that presumably results from a constant integrator sum. If the behavioural weight[13] of the excited neurons is a random function of the distance of the neuron from the electrode tip, the counter model predicts a systematic trade-off between I (which determines the number of neurons excited by each pulse) and P (which determines the number of firings in each neuron within the fixed "time-windows").

The Counter Model and the Data

The P-I trade-offs obtained were fairly systematic (Fig. 5) within certain limits of P and I. None of them, however, were the perfectly straight lines predicted by the counter model. While it was not the purpose of this thesis to evaluate the counter model, it was still necessary to determine if the data were consistant with the model and its major assumptions. If the observed deviations from the model's predictions are consistant enough, it might be necessary to refine the model by adjusting some of the model's assumptions. If too many adjustments are required, the model may no longer be the simplest

[13] Those behaviourally relevant neurons which have a greater effect on the follower neurons may have a greater influence upon the integrator and hence the behaviour. There is no reason to assume that the behaviour weights are all equal, but as long as the weights are not systematically related to the distance from the electrode, then any given I increase should have no greater effect than any other increase of the same magnitude.

explanation of the data. However, if the model can account for most of the data, then consistant differences observed between different groups of curves may be interpreted in terms of the model as implying certain neural events.

One of the main problems with the interpretation of the trade-off data is the variability of the curves. Not only are the curves not straight lines, but also no two curves are the same. In principle, the two determinations of the P-I trade-off for a single behaviour in one animal should be identical. There are several possible reasons why they are not. One is the random variability of the test results which could be due to imprecise relationship between the ESB that an animal receives and the behaviours that ensue. This random variation may stem from myriad sources ranging from the actual excitability of the stimulated tissue, to fatigue, momentary changes in attention and so on. While this deficit in the experimental control of the behaviour might be less in other paradigms (eg. Experiment II, Shizgal, 1975) it's effect on the P-I curves of this experiment should be to introduce funny bumps and random irregularities.

The magnitude of the random variability may be most evident in the difference between the two different determinations of the same trade-off in each animal. The effect of this factor might have been reduced had the animals been tested with all the different intensities before the trade-off curves were run (Edmonds, et al., 1974). The trade-off curves for the ON and for the OFF responses of animal 1A for instance are very close together even though they were determined six months apart. This particular animal was in many ways a pilot animal and fortuitously had been tested with a large range of I's before the

first P-I trade-off was run. No one as yet has determined the exact nature of the influence of experience upon the shape of the P-I trade-off.

It is possible to predict the influence of tissue damage at the electrode tip on the trade-off curves. If the electrodes are allowed to polarize or if the ESB is itself injurious, damage to the neurons close to the electrode would shift the whole trade-off curve to higher I's and lower P's. Although the stimulation was designed, programmed and monitored to minimize this possibility, the systematic curve shifts seen in animals 8A, 18A and 20H could be due to this factor.

There does not seem to be a particular value of P or of I that causes a uniform unusual effect across all animals. Although many of the curves flatten out at low P's and high I's the value of each where this occurs is not uniform for animals or behaviours.[14] It is, therefore, likely that this effect is due to the spatial limitations of the relevant population(s). If it is assumed that roughly the same neural structures are being stimulated in all the animals (as shown by the histology), the lack of a peculiar P indicates that the systems following the activity generated by the ESB are not preferentially sensitive to particular stimulating frequencies.

Although the P-I trade-offs presented here do not eliminate the possibility that each pulse produces more than one AP, when the data are combined with the results of previous studies, this possiblity is rendered unlikely. In studies involving the simultaneous stimulation and

[14] "Behaviours" in the present context will always refer to ON responding and OFF responding.

recording of central neurons, many have shown that .1 msec cathodal or biphasic pulses produce only one AP per neuron when stimulating frequencies up to 15 Hz (P = 70 msec) are used (eg. Rolls, 1971; German and Fetz, 1976 or Fuller and Schlag, 1976). If for some unknown reason, pulses presented at shorter P's than 70 msec were to produce more than one AP per neuron all of the P-I trade-off curves would show a consistant flattening beyond the critical P value. Since the P's at which some of the curves from the different animals flatten were not all the same, it is a reasonable assumption that the pulses did not produce more than one AP per neuron at the lower P's.

It is possible that within a range of short P's, all of the pulses might not produce AP's. If, for example, the P of the ESB was shorter than the RP's of the relevant neurons, then only every n^{th} pulse would fire the neurons (where n is approximately equal to $\frac{RP}{P}$). This effect would appear on the P-I curves as a very steep slope at short P's. Because the RP of any given neuron is relatively constant, increasing the P beyond the RP would allow almost no change in I. Since the trade-off curves do not show this effect, the P-I curves support the assumption that within the range of P's of the P-I curves, each pulse produces one and only one AP.

The justification of the assumption concerning P allows the discussion of the P-I curves to concentrate on the effects of I alone. While the effect of P changes are expected to be reasonably uniform across animals, the effect of changes in the value of I are expected to vary with the location of the electrodes. As noted earlier, the effect of I changes depend to a large extent upon the spatial distribution of the neurons in the region of the electrode tip. When the

electrode tip is near a dense population, I's should be more effective then when the electrode tip is further removed from the same population or when the population is distributed diffusely around the electrode tip. If the membrane thresholds of one particular population of neurons were lower than those of another, the TD would be larger for that population. If the populations were equally distributed in space, more neurons from the low threshold population would be excited by a given I.

It may be possible to determine different effects of I in the trade-off data if these effects cause great enough changes to the curves to be distinguished from the random variability. While the ON and OFF P-I curves for animals 1A, 12H, and 23H are distinctly different, the curves of 8A, 18A are possibly different and the curves for 20H are very similar. Any hypothesis that tried to account for these patterns on the basis of P alone would have to deal with the non-uniformity of the effect across animals. Any hypothesis that tried to postulate differential behavioural weights of downstream collaterals would also suffer the same problem.

Figure 6 shows that the relationship of the average P threshold to the location of the electrode tip is systematic for the ON response but not for the OFF response. If it is assumed that the spread of current around all the electrodes activated roughly the same neural structures (except 1A) and that the effects of P were uniform across animals for any particular behaviour, then it is reasonable to conclude that the difference in the curves for any one behaviour of any one animal reflects differences in either spatial distribution or in membrane thresholds of the excited neurons. The effect of membrane thresholds should be uniform across animals and since the difference between the ON

and OFF curves are different across animals, it is possible to conclude that the spatial distribution of the ON and OFF neurons around the electrode tips are not only different from the two systems, but are also different across electrode locations.[15]

The two-population, two affect theory predicts that although the two populations overlap in the region of the electrode tips, their distributions and densities could well be different. All one-population theories predict that the distribution of neurons underlying the ON response should be identical with the distribution of neurons underlying the OFF response. Even though it might take different amounts of stimulation to produce a given latency of each response, one-population theories would predict that this difference should be uniform across electrode locations. The P-I trade-off data indicate that for the electrode locations studied, this is not true. These data, which imply not only different spatial distributions around any one electrode but also consistant shifts in the distributions with the anterior-posterior changes in electrode location, are difficult to explain in terms of one-population, but very consistant with the two-population two-affect theory.

II. Local Potential Summation (LPS)

The effects of the T-pulses in trains of pulse pairs was found to be highly dependant upon the C-T interval. The presentation and

[15] Although this study was not designed to be a mapping study, the consistancy of the electrode placements and the relationship between mean P and the electrode locations (Figure 6) makes it possible to postulate that the spatial density of the ON system, or it's proximity to the electrode tip, increased along the rostal-caudal axis.

discussion of these effects will be divided into two sections because the mechanisms that best account for these effects depend upon the C-T interval being considered. The Introduction presented the idea that trains of double pulses can be more effective than trains of single pulses because longer C-T intervals allow the neurons to recover from the effects of the C-pulse and fire again to the T-pulse. Double pulse trains with very short C-T intervals should have the same effect as single pulse trains because the T-pulses stimulate neurons in their refractory periods.

This mechanism, however, is unable to account for the higher COP's that were observed when the C-T interval was 0.2 msec. The average percent increase seen over the six repeated measures in each animal are shown in Table 1.[16] When the C-T interval is this short,

TABLE I

Mean T_E's when C-T interval = 0.2 msec.

		Electrode						
		1A	8A	12H	18A	20H	23H	Mean
Behaviour	ON	20.8	7.2	45.5	40.7	65.1	11.1	31.7
	OFF	25.6	66.2	32.1	16.7	20.4	18.3	29.9

there is too little time between the pulses for any neuron fired by the C-pulse to recover its excitability and fire again. The most reasonable

[16] Recall that an increase in the COP reflects the ability of less stimulation to produce the same level of behaviour. The rationale for the calculation of T_E will be developed later.

explanation of these COP increases, consistant with most previous pulse pair studies (see Yeomans, 1975 for a review) is to attribute these increases to the effects of local potential summation(LPS).

Although LPS is a well understood neurophysiological phenomenon (eg. Erlanger and Grasser, 1937) its occurrance around an intracranial electrode requires some explanation. This explanation will be limited to the situation in which two pulses of equal intensity are delivered through the same electrode.

When a single fiber is stimulated with a single, brief, cathodal pulse (the C-pulse) the membrane potential rapidly rises from it's resting level. If the membrane potential rises above threshold, an action potential is generated at the site and propagates down the axon. If the stimulating intensity is insufficient to depolarize the membrane beyond its threshold, the membrane potential generated by the pulse will travel passively only a short distance away from the site of stimulation. In other words, it is a local potential (LP). At the site of stimulation, the stimulation-induced depolarization is counteracted by a reverse flow of ionic current and the membrane potential returns to its resting level within a few tenths of a msec.

If the subthreshold C-pulse (one that creates only a local potential) is followed by a T-pulse of equal size (intensity and duration), the local potential created by the T-pulse can summate with any local potential remaining from the C-pulse. If this summation of local potentials (LPS) is sufficient to depolarize the axon membrane beyond its threshold, an action potential results. The occurrance of sufficient LPS to fire the axon depends upon (a) the magnitude of each LP relative to the threshold, (b) the rate of LP decay and (c) the C-T interval.

(The effects of relative current intensity and C-T interval are illustrated in Figure 8).

To describe how these three factors operate on the tissue, it is necessary to make use of some of the assumptions and concepts developed in the previous section. If the tissue around a monopolar electrode acts as a volume conductor, then neurons as far away as the threshold distance (TD) are fired by a stimulation pulse (a C-pulse). Local potentials are generated in those neurons just beyond the TD. If a second pulse (the T-pulse) of equal intensity follows the C-pulse after a very short interval, there may be sufficient LPS in some neurons to fire them.

If a train of pulse pairs is delivered such that the C-T interval is very short and the P reasonably long[17], then each C-pulse fires neurons out to the TD and each T-pulse fires some neurons beyond the TD, through LPS (Figure 7). The T-pulses do not fire any neurons between the electrode and the TD because these neurons have not had sufficient time to recover their excitability. The limiting outer radius of the region fired by LPS is the distance to which neurons can be fired when the C-T interval is .1 msec.[18] As the C-T interval increases, there is increasing time for LP decay and the outer radius of the LPS region decreases to the TD.

17 The previous and following sections discuss the required P.

18 At this C-T interval, there is no time between the offset of the C-pulse and the onset of the T-pulse. The stimulating pulse is then a single .2 msec pulse. To get an idea of strength-duration, strength-distance relationships, see Ranck, 1975.

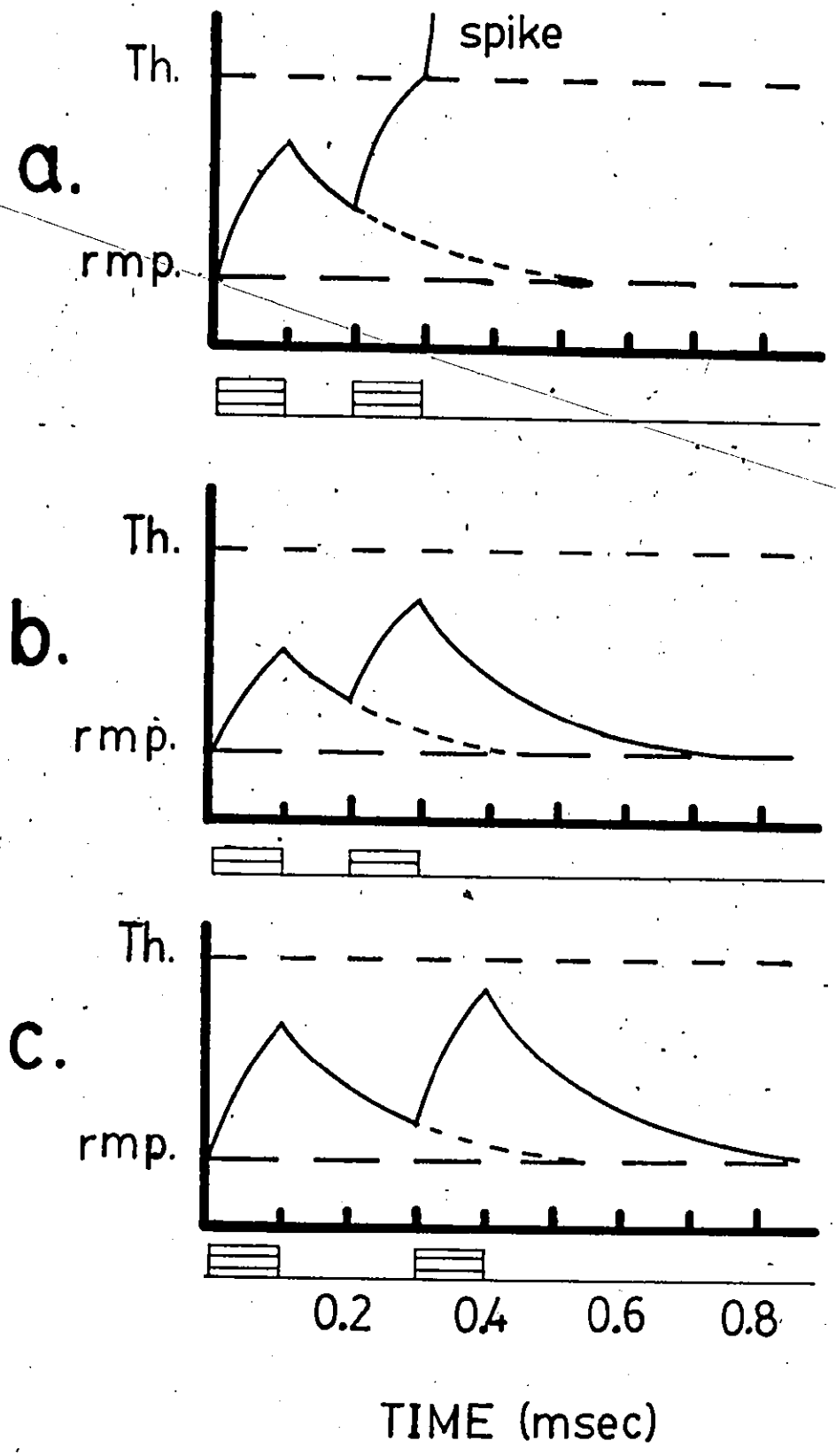
Figure 8. The effects of two equal pulses upon the membrane potential of a hypothetical axon. The ordinate represents the membrane potential, the dashed lines represent the resting membrane potential (rmp.) and the axon's firing threshold (Th). The abscissa shows the time from the onset of the first pulse. The blocks on the line below the abscissa represent the .1 msec stimulating pulses. The height of each block represents the intensity of each pulse relative to the magnitude of depolarization created. A block 3 units high represents a pulse sufficiently strong to depolarize the axon from rmp 3/4 of the way to Th. A block 2 units high represents a pulse intense enough to depolarize a membrane at rmp halfway to Th.

a. The effects of two relatively intense pulses presented with an interpulse interval of .1 msec (C-T interval = 0.2 msec). Note the summation of local potentials that drives the membrane potential beyond Th and leads to the generation of a spike potential.

b. The effects of two relatively weaker pulses when presented at the same C-T interval as in a. Note that the summation of local potentials is insufficient to drive the membrane potential to Th, and therefore, does not lead to the generation of a spike potential.

Figure 8 (con't).

c. The effects of the presentation of two pulses equal in intensity to the pulses used in a., but at a longer C-T interval (0.3 msec). Due to the decay in the local potential generated by the first pulse, the summation between the pulses does not drive the membrane potential to Th and therefore, does not lead to the generation of a spike potential.


spike
Th.
a.
rmp.
Th.
b.
rmp.
Th.
c.
rmp.
0.2
0.4
0.6
0.8
TIME (msec)

At short C-T intervals, the size of the LPS region is some proportion of the size of the C-pulse fired region, because both are based on the relationship between local current intensity and neuronal thresholds. At short C-T intervals, this proportion is non-zero, and more neurons are fired by trains of pulse pairs than are fired by trains of single pulses. If there are neurons in the LPS region that are relevant to the behaviour being studied, then the trains of pulse pairs have a greater effect on the behaviour than the trains of single pulses. The magnitude of this effect depends upon the number of neurons in the LPS region. The greater the number, the greater the effect. This number depends upon the size of the LPS region and the spatial distribution of neurons around the electrode tip. Therefore, the magnitude of the LPS effect at a particular short C-T interval depends upon the spatial distribution of relevant neurons within the LPS region.

In the previous section, the magnitude of the effect of the stimulation intensity was evaluated by determining the COP for each intensity. The present question of concern is the magnitude of the effect of the neurons in the LPS region. This magnitude should be reflected in the difference between the COP of the double pulses (COP_{DP}) and the COP of the single pulses (COP_{SP}). Because the LPS area is proportional to the C-pulse fired area, for any given intensity, the effectiveness of the T-pulses relative to the C-pulses (single-pulses) can be estimated by the ratio of the COP difference to the COP of the single pulses. Multiplying this value by 100 converts it to a percentage score. Therefore, the relative T-pulse effectiveness (T_E) for the C-T interval = 0.2 msec was always evaluated by the expression:

$$T_E = \frac{COP_{DP} - COP_{SP}}{COP_{SP}} \times 100$$

The T_E's for ON and OFF responding were determined six times in all six animals for C-$\bar{T}$ interval = 0.2 msec. This value (0.2 msec) was chosen to estimate the maximum effect of LPS. Because LPS was expected to reflect the spatial distribution of the relevant neurons relative to the electrode tips, the T_E's were expected to be different for different electrode placements. The six measures of T_E for each animal and each behaviour, on the other hand, were expected to be the same, except for random variability. It seemed advisable, therefore, to conduct an analysis of variance (ANOVA) that used the variability of the six repeated measures as the error term.

The ANOVA revealed that the variability between subjects was significant ($F = 3.697$, $df = 5,25$, $p<.012$) and that the interaction between subjects and behaviours was also significant ($F = 11.248$, $df = 5,25$, $p<.00001$). No other testable main effects or interactions were significant ($p<.25$).[19] It is interesting to note that the overall difference between the average T_E for the two behaviours was only 2%. These results indicate that there was no uniform difference in the T_E of the two behaviours across all electrodes, and that the value of T_E depended upon both the particular electrode and the particular behaviour.

Post hoc tests revealed that for animal 8A, the electrode placement revealed significantly larger T_E's for the OFF response than

[19] For the complete source table for the ANOVA, please see the Appendix.

for the ON response (Tukey HSD, 2-tailed, $p<.01$) and that for animal 20H, the electrode placement showed significantly higher T_E's for the ON response than for the OFF response (Tukey HSD, 2-tailed, $p<.02$).

While the interpretation of the T_E's at C-T interval = 0.2 in terms of LPS is not the only possible one, it is the most reasonable because there are very few other neural events that have this time course.[20] The data indicate that LPS for ON responding is not necessarily equal to the LPS for OFF responding. It is possible for an LPS difference to be due to a difference in the behavioural weights of the neurons in the LPS region, or a difference in spatial distribution of the neurons in the LPS region. Because the direction of the difference was opposite for 8A and 20H, it is unlikely that the difference could be due to differential behavioural weights, and so it seems reasonable to conclude that the difference observed in LPS is due to a difference in the spatial distribution of the neuron populations in the LPS region.

This difference in spatial distribution implies that the neuron population subserving the OFF responding is not the same as the population subserving the ON response. Although the difference in T-pulse effectiveness was significant in only two animals, the data from the other four animals do not imply that the populations subserving the two responses were the same. The screening of the animals biased the selection of electrode placements towards those placements where the

[20] The second important indicator of LPS, its decay, will be shown in the next section.

stimulation produced roughly equal, mixed effects. The intensities at which the pulse pairs were tested were chosen on the basis of observing COP_{SP}'s for the two behaviours within a relatively limited range. The observation of large differences despite these procedures to minimize them and the lack of a systematic relationship between the differences and the electrode placements implies that the differences might be due to regional non-homogeneities in the spatial distribution.

If the neuron populations relevant to each behaviour were not distributed homogeneously with respect to the electrode tip, then the addition of a unit area of tissue would not be expected to cause a uniform shift in the COP. This should be true whether the area is added by LPS or a unit increase in the stimulation intensity. This hypothesis was tested in two ways. The relationship between COP increase caused by an intensity increase and the COP increase caused by the addition of the T pulse at 0.2 msec was determined first for a single intensity in each animal and second, for eight different intensities in one animal.

The effect of the intensity increase in each animal was evaluated by calculating the percent change in COP following a twenty-five percent increase in intensity above the intensity used in the double pulse tests. The correlation between the COP increase caused by intensity increase and the COP increase caused by LPS was found to be .78 for turning on ($p<.05$, two tailed) and .83 ($p<.05$, two tailed) for turning off. These correlations imply a systematic relationship between the effects of an intensity increase and the presentation of T-pulses at C-T = 0.2 msec. Although this is consistant with the

hypothesis that both effects are due to the firing of additional neurons, these correlations do not say much about whether the differences in LPS effects between animals are due to regional non-homogeneities.

If regional non-homogeneities exist around the electrode tip then the amount of LPS should be different for different intensities. Furthermore, the amount of T_E due to LPS at each intensity should correlate with the percent increase in COP's caused by a twenty-five percent increase in the intensity. This hypothesis was tested at eight different intensities in a single animal.[21] It was found that the correlation between the percent COP increase caused by the intensity increase and the percent COP increase caused by the T-pulses was .80 for turning on and .84 for turning off. These correlations are again consistant with the hypothesis that the LPS effects and the effects of intensity increases are due to the firing of additional neurons. The range of the T_E's (4 - 73% for turning on, 4 - 64% for turning off) revealed that there were considerable differences between the amount of LPS at some intensities and the amount at other intensities. This is consistant with the hypothesis that fluctuations in T_E at C-T = 0.2 msec are due to regional non-homogeneities.

One-population theories of the two behaviours would predict that there should be a high correlation between the T_E for turning on and the T_E for turning off. The correlation between the T_E's at the eight different intensities in the one rat tested was found to be .38 and the

[21] This animal (23H) was one of the four for whom the difference in T_E at C-T = 0.2 msec was non-significant.

correlation between the mean T_E's for the six animals who were tested repeatedly was found to be -.14. These values are clearly not the high correlations that would be expected from one population theories, but they are of sufficiently low magnitude as to be consistant with the two population hypothesis.

In summary, there were significant differences between T_E for turning on and T_E for turning off when the C-T interval was equal to 0.2 msec. The most reasonable explanation of the T_E at this C-T interval is in terms of the effects of the neurons fired by LPS. This interpretation is supported by correlations between T_E's at this interval and COP increases that are caused by increasing the stimulation intensity. These correlations imply that fluctuations in the magnitude of the LPS effect are related to regional non-homogeneities in the distribution of relevant neurons around the electrode tip. The absence of correlation between the T_E's for the two behaviours for two of the animals, when combined with the LPS interpretation of the data, all lead to the conclusion that the two behaviours observed in this paradigm are due to the excitation of at least two relevant but functionally distinct neuron populations.

III. Refractory Period Estimates

The previous section of the Results and Discussion dealt with the T_E when the C-T interval was equal to 0.2 msec. This section deals with the T_E's when the C-T interval was equal to the other eight values

tested.[22] The present T_E was calculated by exactly the same formula as was used for C-T interval = 0.2 msec although the rationale is somewhat different. This formula:

$$T_E = \frac{COP_{DP} - COP_{SP}}{COP_{SP}} \times 100$$

is the same formula that Yeomans (1975) developed, except that the ratio is multiplied by 100 to provide a percentage score. The values for the single-pulse and double-pulse conditions are inverted in position with respect to Yeomans' formula because $P = \frac{1}{F}$, and therefore, COP is equivalent to $\frac{1}{FT}$.

The T_E formula is somewhat easier to grasp intuitively because it compares the change in the COP_{DP} at any given C-T interval to the constant COP_{SP}. For example, consider the two extremes of T_E. When the C-T interval does not exceed the RP's of any of the relevant neurons, then exeluding the effects of LPS, the T-pulses should not fire any neurons, the COP_{DP} will be equal to the COP_{SP} and so the T_E = 0.0%. When the C-T interval exceeds all of the RP's of the relevant neurons, the T-pulse will fire all of the same neurons as were fired by the C-pulse. The behavioural effect of these T-pulses should, therefore, be the same as the behavioural effect of T-pulses introduced halfway between the C-pulses. Since this is essentially a halving of the P, the COP_{DP} is expected to be double the COP_{SP} and produce a T_E = 100.0%

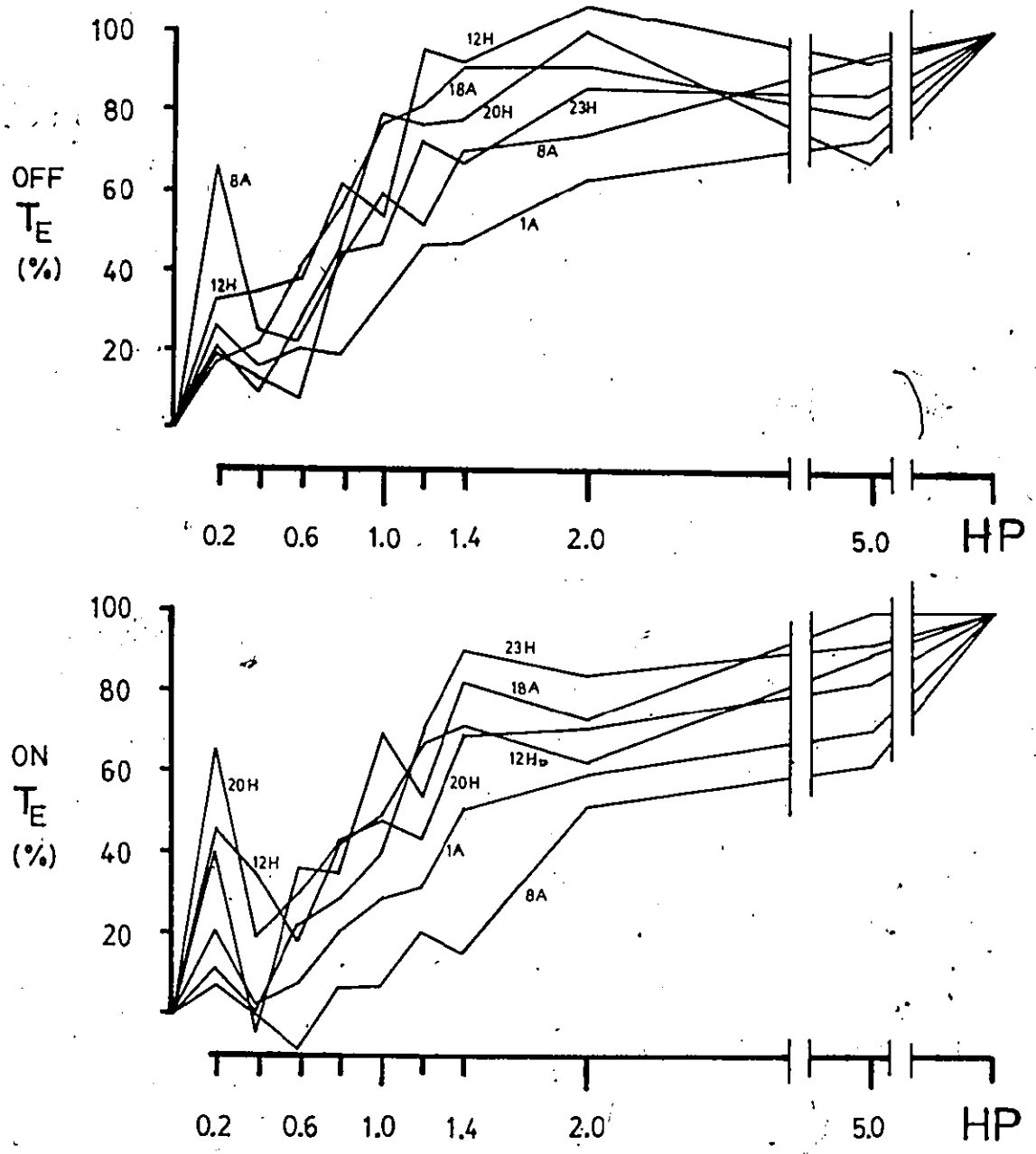
[22] All figures in this section will, however, present the T_E's at C-T = 0.2 msec. Each graph will therefore illustrate the generally lower T_E's at C-T = 0.4 which are necessary for the LPS interpretation of the T_E's at C-T = 0.2 msec.

Figure 9 presents the relationship between T_E and C-T interval of all six electrode placements for both behaviours in a form designed to reveal the variability of the curves and each behaviour. This format also reveals a general overall pattern of changes in T_E with C-T interval that is consistant with Yeomans' data (1975, 1977).

All of the curves can be loosely segmented into three phases. The first phase is a peak in T_E at 0.2 msec (as discussed in the previous section of the Results and Discussion) which generally declines to a low T_E at 0.4 msec. The second phase is a relatively rapid increase in T_E over C-T intervals of 0.6 to 1.2 or 1.4 msec. The third phase of the curves is generally a very gradual increase in T_E from 1.4 to 5.0 msec. The lines drawn between T_E at 5.0 msec and T_E = 100% at HP (Half-Period) accentuate the discrepancy between the T_E at 5.0 msec and the effect of halving the period (by definition).

Theoretically, the C-T intervals over which the curves rise to an asymptote should define the range of refractory periods of the neurons relevant to the two behaviours. A difference between the ranges of these intervals for the two behaviours would therefore, serve to indicate a difference in the neuron populations relevant to each behaviour. Unfortunately, the C-T interval range over which T_E rises rapidly, varies considerably from animal to animal. To illustrate this point, the C-T interval that led to an arbitrary T_E (50%) was determined for each animal on both behaviours. These values presented in Table II merely represent a convenient condensation of the curves and have no particular theoretical significance other than their illustration of the variability between the curves of the different animals.

Figure 9. The mean values of T_E at each C-T interval. Each data point represents the mean of the six repeated measures and each curve on a given set of axes derives from the data of a different animal. The alphanumeric character closest to each curve indicates the animal number and the electrode location (A = left LH, H = right LH). One set of axes shows the curves derived from latencies to turn the ESB on (ON T_E) and the other set shows the curves derived from latencies to turn the ESB off (OFF T_E).


OFF
T_E
(%)
100
80
60
40
20
12H
18A
20H
23H
8A
8A
12H
1A
0.2
0.6
1.0
1.4
2.0
5.0
HP
ON
T_E
(%)
100
80
60
40
20
23H
18A
12H
20H
20H
12H
1A
8A
0.2
0.6
1.0
1.4
2.0
5.0
HP

C-T Interval

TABLE II

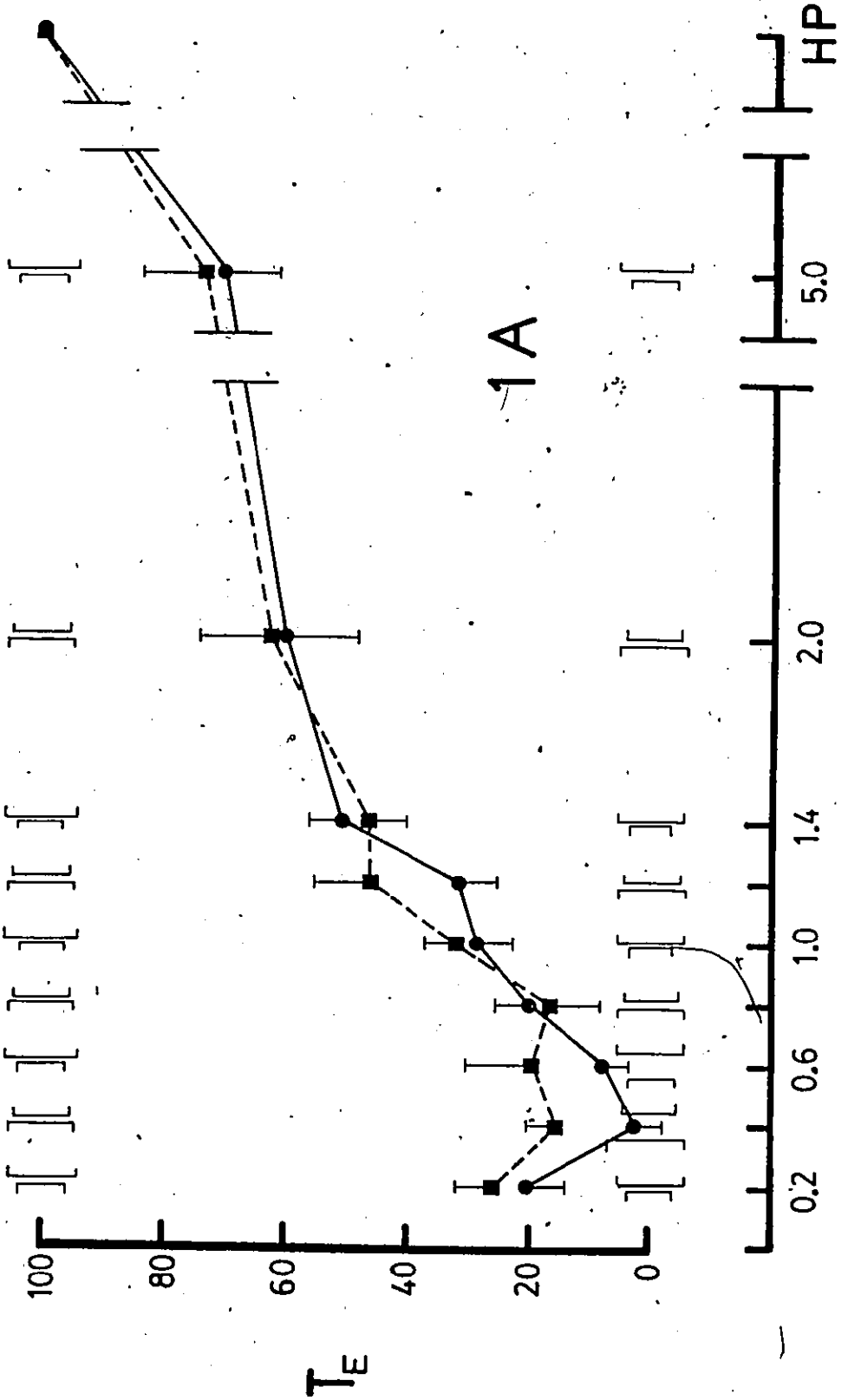
C-T intervals where T_E = 50%

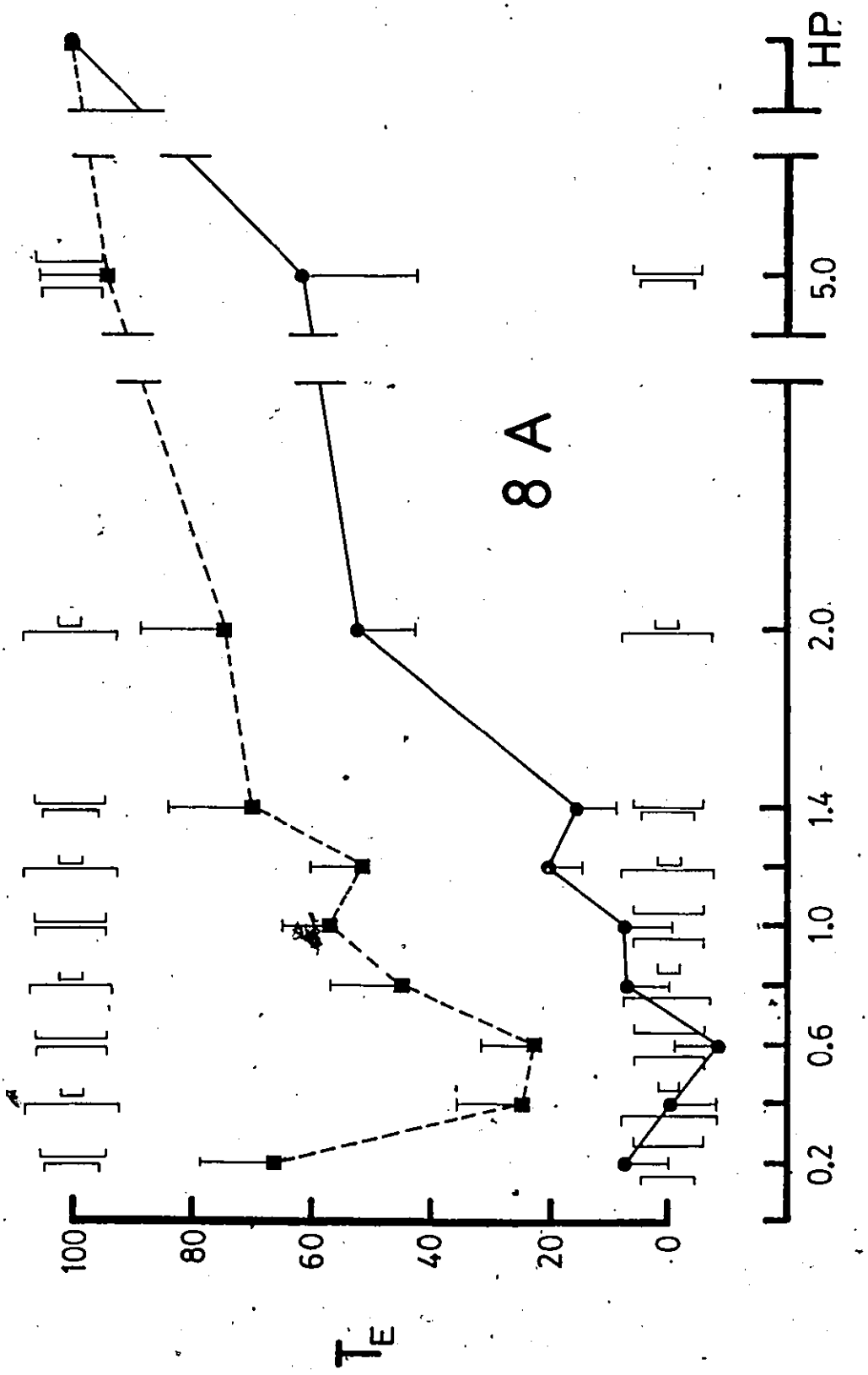
Behaviour	Electrode	1A	8A	12H	18A	20H	23H
	ON	1.40	1.96	1.00	.80	1.25	1.07
	OFF	1.55	.66	.70	.73	.73	1.03

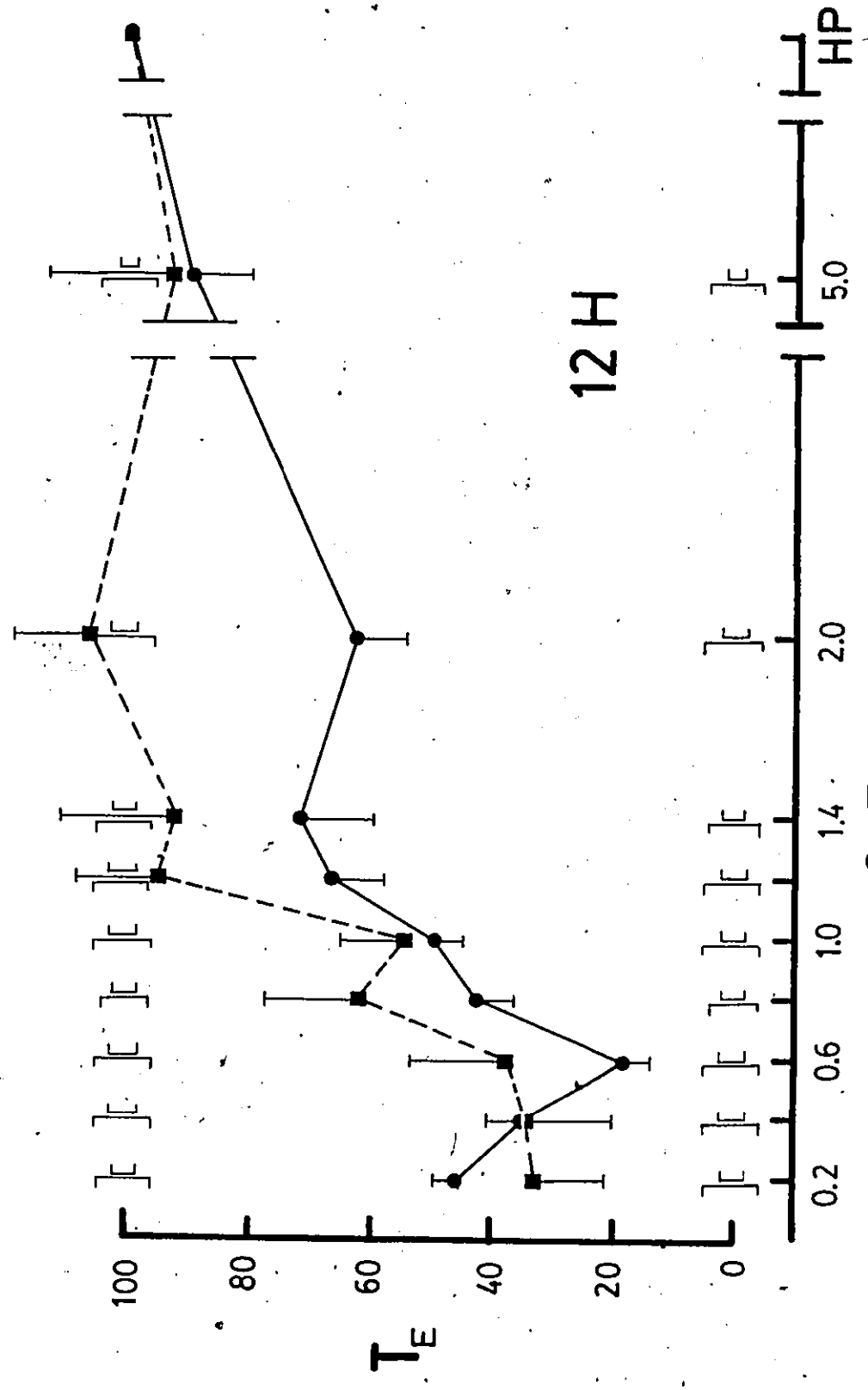
It is the variability which makes it difficult to make any confident estimates of the refractory periods of the neurons underlying the two behaviours. This variability also makes it unrealistic to maintain that these data provide a means of discriminating the relevant neuron populations on the basis of their refractory periods.

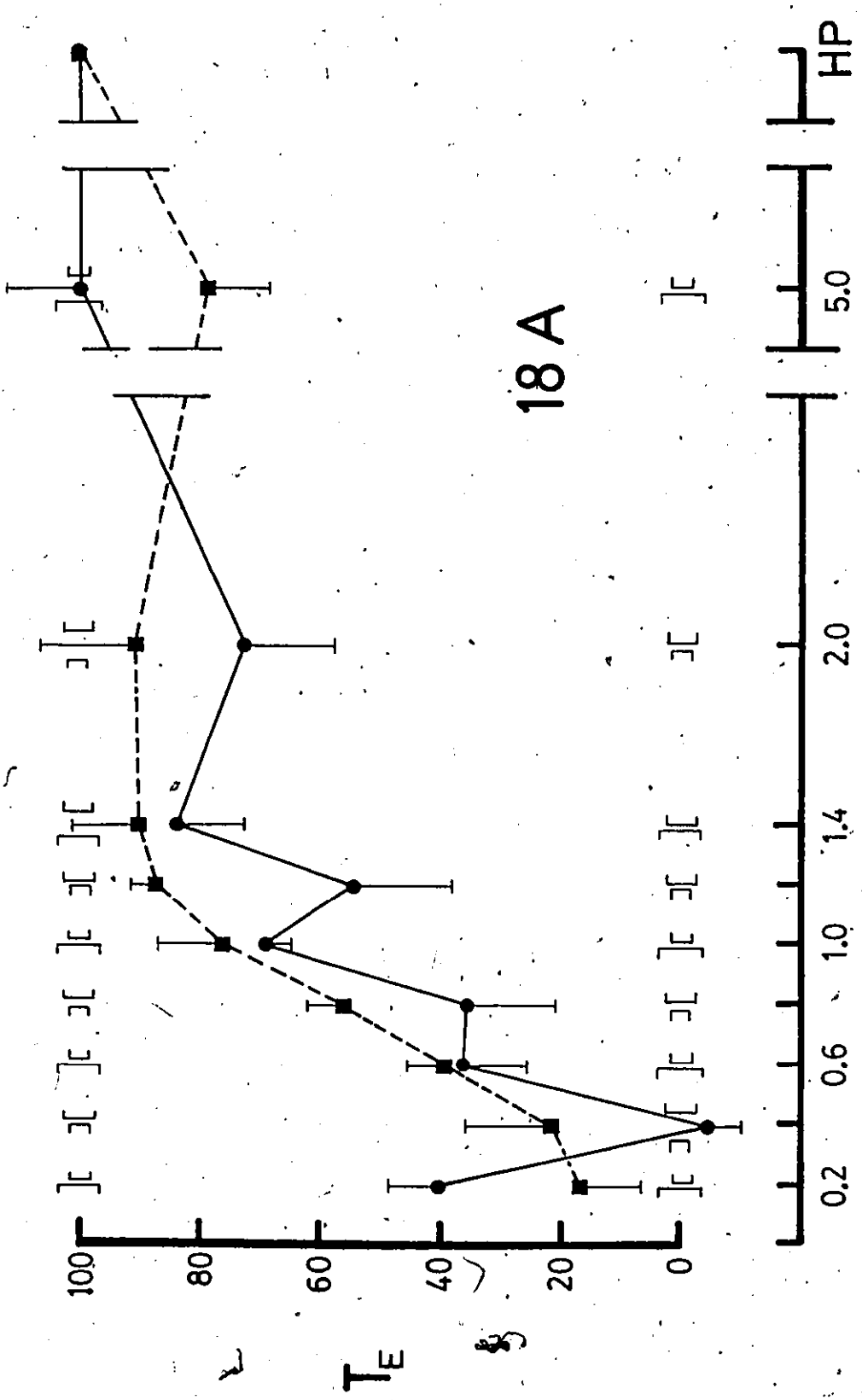
Although there are many possible factors which could have contributed to the differences between the animals, an examination of the variability within each animal's data might reveal whether it is worthwhile to consider the other, more meaningful factors. Figure 10 presents the same T_E versus C-T interval curves as were presented in Figure 9 but groups the curves differently. Plotted on each set of axes are the T_E curves for turning on and turning off derived from the data of each animal. This format simplifies the comparison of the T_E curves for the two behaviours of each animal and provides room for the illustration of the variance associated with each mean value (the error bars indicate one standard error of the mean).

Figure 10. Within animal comparisons of T_E across behaviours. Each set of axes contains the mean T_E of the six repeated measures on each animal and reveals the similarities and differences of the curves for the two behaviours. The squares joined by dashed lines show the mean T_E's (of the six repeated measures) for the OFF response. The circles joined by solid lines show the mean T_E's (of the six repeated measures) for the ON response. The large alphanumeric character denotes the animal number and electrode location (A = left LH, H = right LH). the error bars on the mean T_E's indicate one standard error of the mean (SEM) in one direction. Since the SEM is symmetrical about the mean, the SEM in the opposite direction was deleted for clarity. The left-facing brackets (]) around T_E = 0 and T_E = 100% indicate plus and minus one SEM of the COP_{SP} for the OFF response and right-facing brackets ([) indicate plus and minus one SEM of the COP_{SP} for the ON response.


1A
T_E
100
80
60
40
20
0
0.2
0.6
1.0
1.4
2.0
5.0
HP
C-T Interval


8 A
T_E
100
80
60
40
20
0
0.2
0.6
1.0
1.4
2.0
5.0
HP
C-T Interval


12 H
T_E
100
80
60
40
20
0
0.2
0.6
1.0
1.4
2.0
5.0
HP
C-T Interval


18 A
T_E
100
80
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40
20
0
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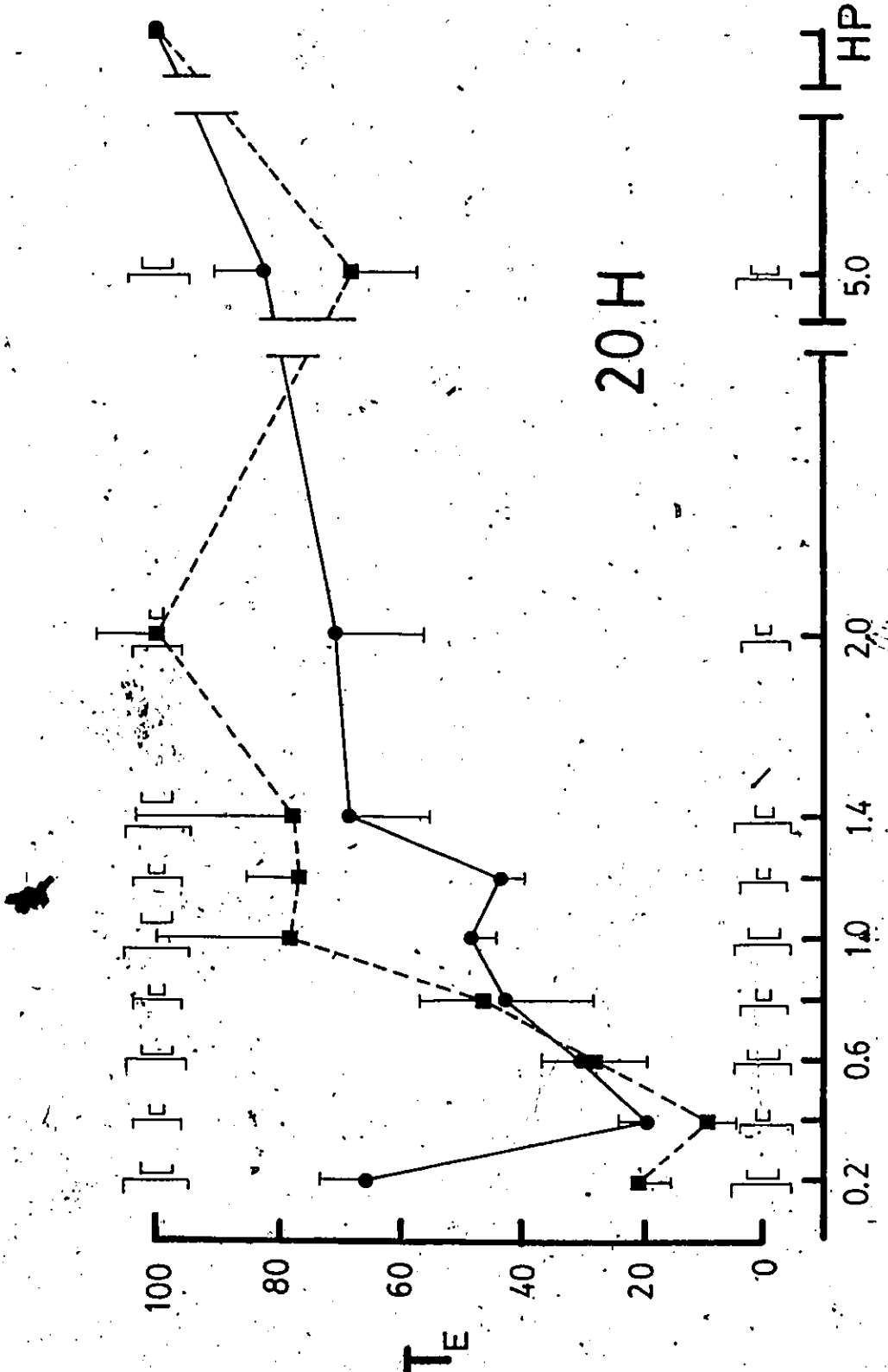
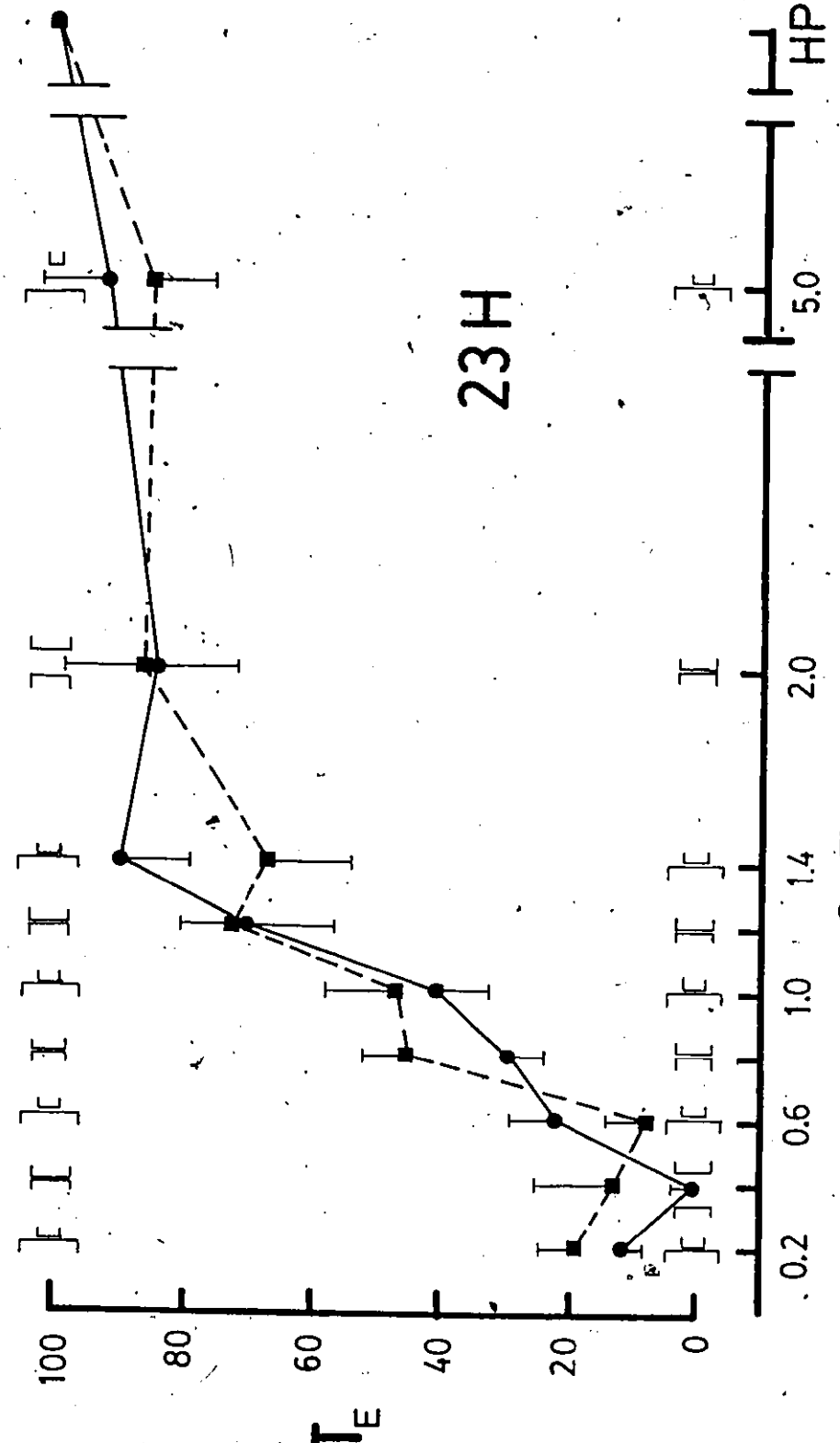
1.0
1.4
2.0
5.0
HP
C-T Interval


20 H
T_E
100
80
60
40
20
0
0.2
0.6
1.0
1.4
2.0
5.0
HP
C-T Interval


23H
T_E
100
80
60
40
20
0
0.2
0.6
1.0
1.4
2.0
5.0
HP
C-T Interval

It is reasonably clear that the variability of the individual T_E determinations[23] was rather large. Since the repeated measures on each animal involve the repeated testing of the ESB's effect on the smae neuron populations, and since the variability of these T_E's was so high, factors which reduced the consistancy of these measurements could account for the between animal variability. Thus, the differences between the T_E's of different neuron populations could well be unrelated to actual differences between the excitability characteristics of the neuron populations themselves.

The primary purpose of the present study is the attempt to discriminate the neural populations subserving the turning on and the turning off of the ESB. One of the implicit assumptions of this study is that a given area of the brain subserves the same function in almost all of the animals of a given species. Therefore, if the electrical stimulation of a certain area of the brain produces the same behavioural effect in a number of animals, it is assumed that the stimulation acts on the same neural structure in all animals.[24] The concept that the refractory periods of the neurons of the relevant neural structure should be the same across animals received empirical support from the data of Yeomans' (1975, 1977). The T_E curves which derived from comparisons of frequency thresholds for self-stimulation were consistant

[23] The error bars displayed around T_E = 0 and 100% indicate the variability of the two COP_{SP} determinations of each day when compared to the mean COP_{SP} of the day. These error bars indicate not only the variability associated with repeated determinations of a COP for any given stimulation condition but also the variability associated with the denominator of the ratio used to calculate T_E.

[24] The validity of this assumption depends to a great extent upon the definition of the phrases "a given area of the brain" and "neural

across his nine placements, one of which was not in the diencephalon. In the present study, the specific assumption is that the neuron populations subserving the ON responses of each of the animals belong to the same neural structure(s) in all the animals. This same assumption applies to the neuron populations which subserve the OFF response.

The statistical analysis of the data therefore, concentrated on the role of three factors on the T_E: the C-T Intervals between 0.4 and 5.0 msec, the behaviours (turning on versus turning off) and the repeated testing. The statistical analysis was a 3-way ANOVA which treated the repeated measures as a fixed factor.[25] The main effect of the C-T interval was significant ($F = 55.48$, $df = 7,35$, $p < .00005$), statistically confirming the significance of the large changes in T_E with C-T interval that are apparent in the data. There was no significant main effect of the repeated measures ($F = .337$, $df = 5,25$) and no significant interaction between the repeated measures and the behaviours ($F = .817$, $df = 5,25$, $p < .55$), the C-T interval ($F = 1.18$, $df = 35,175$, $p < .24$) or both ($F = 1.22$, $df = 3,175$, $p < .20$). These indicate that changes in the T_E's which occured over the repeated testing did not introduce systematic variance to the T_E's.

structure". This assumption could well be tenuous if applied to a large area of the brain such as the diencephalon but might be tenable in relation to a small area of the brain, such as a millimeter length of the medial forbrain bundle at the level of the LH or to the electrode placements used in this study.

[25] The entire source table for the ANOVA is presented in the Appendix.

The F ratio for the main effect of behaviours was quite low ($F = 6.24$, $df = 1,5$, $p < .055$) indicating that it would be hazardous to say that there was a difference between the T_E's for the two behaviours over all the C-T intervals. This result is quite reasonable and apparent in the data. At short C-T intervals (eg. 0.4 msec) the T_E for both behaviours should be quite low and for very long C-T intervals (eg. 50 msec) the T_E for both behaviours should be quite high. If there had been a factor which caused large, uniform differences in T_E's for the two behaviours, this F ratio would be the one to show it. A difference in the refractory periods of the neuron populations subserving the two behaviours would be indicated in the T_E curves by a systematic divergence in the C-T interval range where T_E is rising and possibly a convergence of the curves towards equal values in the range of the long C-T interval. If this effect was great enough, the F ratio for the interaction between C-T interval and behaviour would be large. It was not ($F = 1.9$, $df = 7,35$, $p < .096$). These statistics confirm the intuitive impressions of the data that are gained from the graphs. That is, given the observed variability of T_E, small differences that might exist between the refractory periods of the neurons subserving the ON and OFF responses could not be observed.

If for some reason, the experimental procedure introduced random errors into the COP's , it is possible that another measure of COP's might result in lower variance, thereby increasing the power of the statistical tests. One of the problems with the 15 sec latency was that some L-P curves were not strictly monotonic in that range. Occasionally an L-P curve would cross the 15 sec latency two or three

times. When this occured, the average COP was used in the calculations. Since it was possible that these multiple crossings might have introduced unnecessary variance into the data, it was decided to take a second set of COP's from the L-P curves of each animal at the latency which showed the fewest multiple crossings. A comparison of Figure 11A and Figure 11B reveals that the overall appearance of the curves for all the animals was not meaningfully changed by this procedure.

A second ANOVA performed on the second set of data revealed that the F ratio for the main effect of behaviour ($F' = 7.13$, $df = 1,5$ $p < .044$) and the F ratio for the interaction between behaviour and C-T interval ($F' = 2.65$, $df = 7.35$, $p < .026$) were larger than the comparable F's of the previous ANOVA.[26] However, since both sets of COP's were derived from essentially the same data, the probabilities of α error given for each F ratio are no longer accurate. The systematic increase in F ratios seemed to indicate that the use of procedures that reduced the error variance might improve the probability of being able to discriminate the two behaviours on the basis of their RP's. The F ratio for the interaction between C-T intervals and behaviours again relates to the degree of convergence and divergence of the T_E curves of the two behaviours and therefore, given the experimental objectives this F is the most important statistic of the analysis.

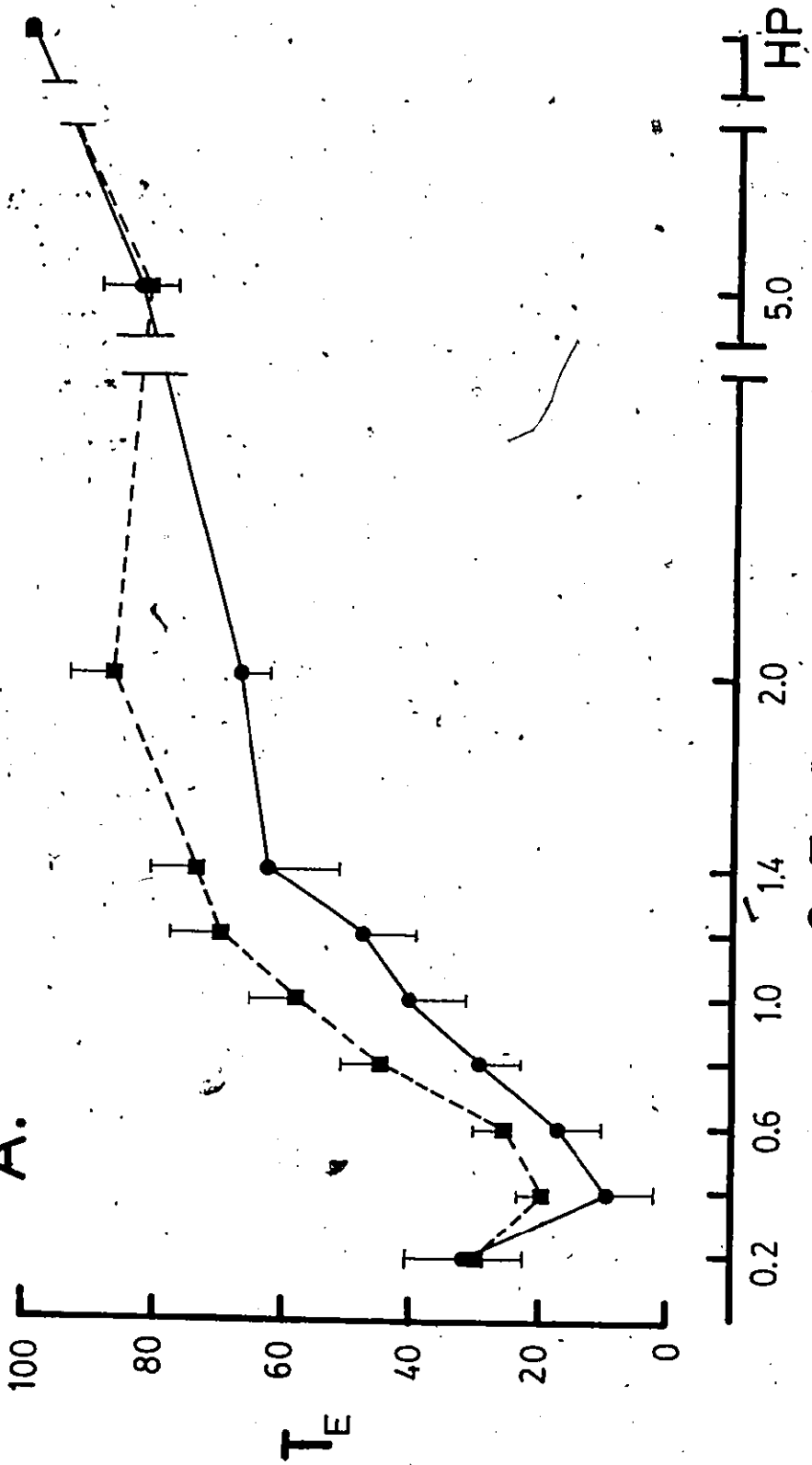
If the significance of the F ratios were to be considered at an α level of .05, and if the second ANOVA had been the only ANOVA conducted, the F ratio for the interaction between C-T interval and

[26] For the full source table of this ANOVA, please see the Appendix.

Figure 11. Mean T_E's for each C-T intervals for all six repeated measures on all six animals. As in previous figures, the squares joined by dashed lines again indicate the T_E's for the OFF response while the circles joined by the solid lines indicate the T_E's for the ON response. The error bars indicate one standard error of the mean, the variability of the means of the six animals.

A. The curves of T_E for ON and OFF responding when the COP's were derived from latencies equal to 15 sec.

B. The curves of T_E for ON and OFF responding when the COP's were derived from the latencies that had the fewest multiple crossings of the P-L curves.


A.
100
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T_E
0.2
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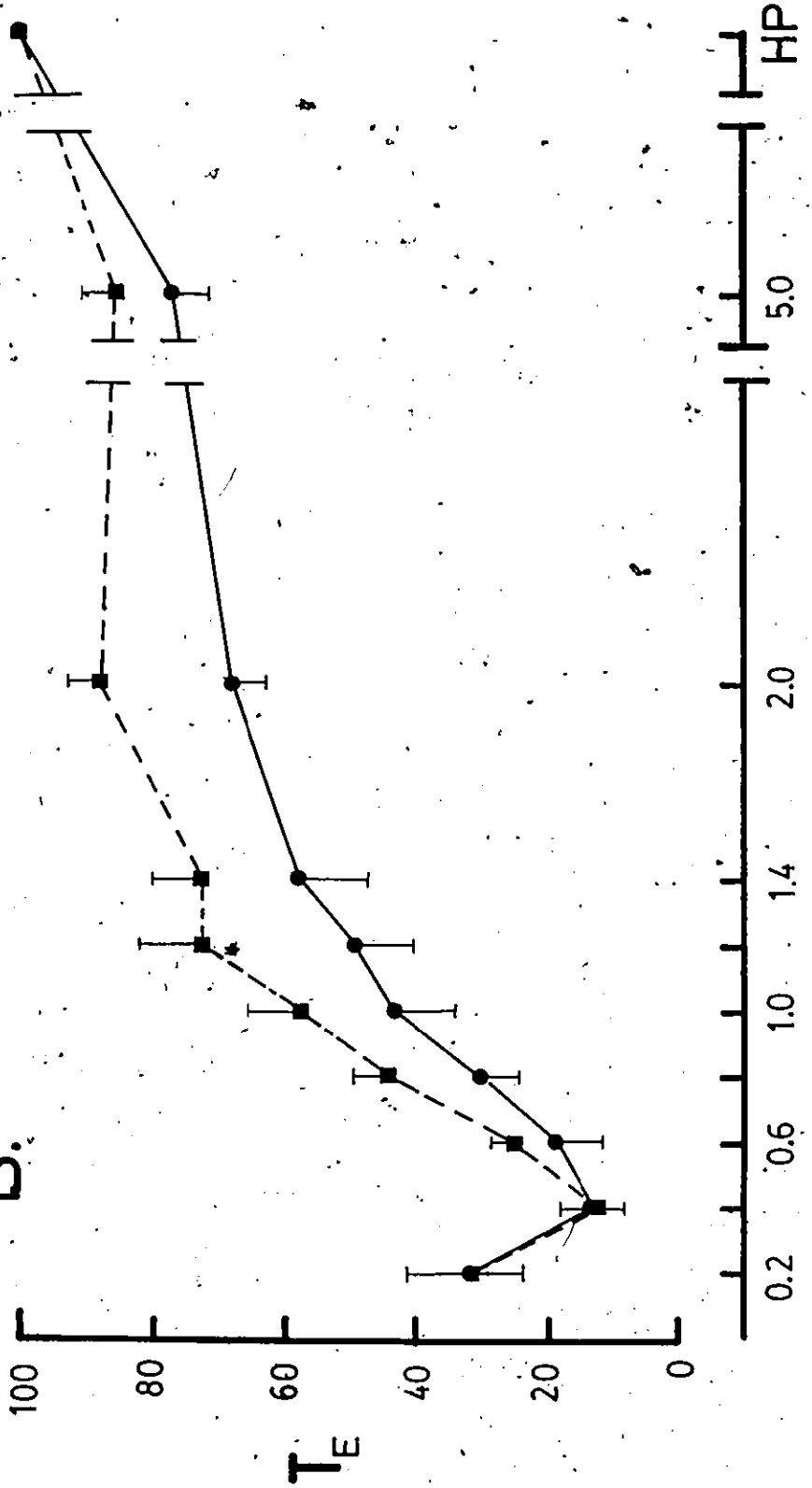
2.0
5.0
HP
C-T Interval


B.
T_E
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5.0
HP
C-T Interval

behaviour would be considered to be statisticaly significant. Because two ANOVA's were conducted on data derived from the same P-L curves, the probability of a given F value derived from the F distribution is no longer accurate. Even if it is assumed that the two ANOVA's were independent, the adjustment of the F probability would bring the p of the interaction F ratio marginally above the liberal α level of .05. The data therefore, do not justify a confident conclusion regarding either the similarity or difference in the RP's of the neurons relevant to the two behaviours.

Furthermore, a visual comparison of the curves that showed the "significant" interaction (Figure 11B) with the curves which showed a "non-significant" interaction (Figure 11A) reveals that these two sets of curves show very little difference in the degree of convergence and divergence. This indicates that the change in the F ratio between the two ANOVA's could well be largely due to a change in variability. Indeed, a comparison of the denominator mean squares (MS) of these two F ratios indicates that the denominator MS was 35% larger for the first ANOVA then it was for the second. The fact that the change in the critical F ratio was caused largely by a change in variance increases the likelihood that the T_E curves for the two behaviours could not be discriminated largely because of excess variance in the COP measuring procedure.

In summary, the frequency scaling methods proposed by Yeomans (1975) were used to examine the RP's of neurons relevant to the turning on and turning off of ESB. They resulted in estimates of T-pulse effectivenesses that closely resembled both the post-stimulation

excitability cycles of individual axons and the previous estimates of T-pulse effectivenesses using the frequency scaling methods (Yeomans, 1975, 1977, Dennis, Yeomans and Deutsch, 1976). Unfortunately the relationship between T_E and C-T interval was not constant across animals for either behaviour, nor was the relationship between the T_E's for the two behaviours at any given C-T interval consistant across the animals. The high variability of the T_E's at any given C-T interval for any given behaviour was probably greater than the variability that might have been observed had other procedures been used (eg. Experiment II, Shizgal, 1975; Yeomans, 1975, Dennis, Yeomans and Deutsch, 1976), but the difficult and protracted nature of these procedures must be weighed against the conclusiveness of the expected data. The slow rise of the T_E curves in this and previous studies indicates that there may be a broad distribution of RP's within the neurons that subserve the turning on and turning off of ESB. This means that only those measures which produce low enough variance to lead to powerful statistical comparisons could be expected to resolve any possible differences between the relevant neuron populations on the basis of their RP's. The experimental procedure used in this study did not lead to T_E's with low variance and no differences between neuronal RP's could be confidently distinguished.

General Discussion

The present study provides additional evidence that multiple behavioural effects of ESB through single electrodes are not necessarily due to the activation of a single generalized system. Evidence from concurrent parametric manipulations of three parameters of the ESB converges upon the general conclusion that the neuron population which subserves the turning on of LH ESB is not the same as the neuron population which subserves the turning off of the LH ESB.

Not all of the parametric manipulations of the ESB were found to result in conclusive evidence for the two-population hypothesis. When T-pulses were delivered at C-T intervals of 0.4 - 5.0 msec, the relationship between T_E and C-T interval was reasonably close to the values expected from post-stimulation excitability cycles of individual neurons. Unfortunately, there was a great deal of variance associated with the mean T_E's at each C-T interval and it was not possible to discriminate between the mean curves of the two behaviours.

To determine if the arbitrary choice of the 15 sec latency as a constant output weight had introduced unnecessary variance to the T_E's, all of the T_E's were recalculated for what appeared to be the most stable latency of each animal. The ANOVA on these new data indicated that the reduction in T_E variance which resulted from the changes in the latencies increased the F ratio. Because the significance of the difference between the T_E's of the two behaviours remained equivocal, it was still not possible to conclude that the data from most of the C-T intervals definitely supported the two-population hypothesis exclusively.

There are some indications from other studies that the RP's of central neurons may not be all that dissimilar for neuron populations subserving different responses. Yeomans (1975, 1977) estimates of the RP's of neurons involved in self-stimulation lay between 0.6 and 1.2 msec for placements in the MFB and near the Locus Coeruleus. When Dennis, Yeomans and Deutsch (1976) studied the RP's of neurons subserving the operant escape from intermittant stimulation of the medial lemniscus, they estimated that the RP's lay between 0.6 and 1.0 msec. Perhaps it is not too surprising that the T_E values in the present experiment were not found to be highly different for the two responses.

The data from the other parametric manipulations of the ESB provide much more conclusive evidence for the two-population hypothesis. There were large differences in the T_E's at C-T = 0.2 msec that were shown to be most reasonably attributable to differences in the effects of LPS around each electrode. Since these differences are most reasonably accounted for by differences in the spatial distributions of the neuron populations relevant to the two behaviours, the conclusion is that there were two functionally distinct relevant neuron populations around each electrode but that they were to some extent, spatially overlapping. This conclusion is supported by the findings that the changes in COP that result from presenting T-pulses at C-T = 0.2 msec and a given intensity correlate highly with the COP changes observed when the current intensity is increased by 25%.

The interpretation of the changes in COP in terms of spatial distributions assumes that the differences between the LPS effects of the different electrodes are due to variable spatial densities of

neurons in the LPS region which result from regional non-homogeneities of the neuron populations' spatial distribution. Although only one animal was tested to provide evidence for this assumption, the correlations between the LPS effects and the effects of current intensity increases for this one animal were very consistant with this assumption.

Evidence for regional non-homogeneities of the individual neuron populations and for the differences in spatial distributions of the two populations of neurons relevant to the two behaviours was also provided by the P-I trade-offs. Changes in P were shown to be systematic over a large range of values while the effects of changing the I seemed to depend upon the spatial distribution of the relevant neurons themselves. The trade-off curves indicated that the spatial distributions of the relevant neurons were different. The consistant relationship between the neuroanatomical location of the electrode tip and the P-I trade-off curves for the ON response but not the OFF response was a further indication of the independance of the neuron populations.

The general conclusion of this study is based on the overall picture presented by these convergent lines of evidence. These lines all involve the parametric manipulations of ESB to determine constant output functions. The evidence presented here indicated that the neuron populations which subserve the turning on and turning off of LH ESB are functionally distinct but neuroanatomicly overlapping in the region of the electrode tip.

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Appendix: Source Tables of ANOVA's

A. ANOVA on T_E's at C-T interval = 0.2 msec.

Source	df	MS	F
Behaviours (B)	1	41.41	.138
Error (B X Repeated Measures (RM))	5	299.50	
Electrode Placements (E)	5	1387.76	3.697*
Error (E X RM)	25	375.89	
B X E	5	3796.35	11.248**
Error (B X E X RM)	25	337.51	

* $p < .02$

** $p < .00001$

B. ANOVA on T_E's for C-T intervals of 0.4 msec to 5.0 msec when the T_E's were derived from 15-sec latencies.

Source	df	MS	F	p<
C-T interval (CT)	7	44,732.8	55.486	.00005
Error (CT x Subject (S))	35	806.2		
Repeated Measures (RM)	5	492.9	.337	.89
Error (RM x S)	25	1,462.4		
Behaviours (B)	1	23,527.7	6.244	.055
Error (B x S)	5	3,767.9		
C-T x RM	35	859.2	1.181	.24
Error (CT x RM x S)	175	727.8		
CT x B	7	1,010.4	1.917	.096
Error (CT x B x S)	35	527.2		
RM x B	5	1,027.2	.817	.55
Error (RM x B x S)	25	1,256.5		
CT x RM x B	35	562.0	1.225	.20
Error (CT x RM x B x S)	175	458.8		

C. ANOVA on T_E's for C-T intervals of 0.4 msec to 5.0 msec when the T_E's were derived from the latencies with the fewest multiple crossings.

Source	df	MS	F	P<
C-T interval (CT)	7	45,260.2	45.859	.00005
Error (CT x Subjects(S))	35	986.9		
Repeated Measures (RM)	5	1,132.6	1.049	.41
Error (RM x S)	25	1,080.1		
Behaviours (B)	1	23,857.4	7.131	.044
Error (B x S)	5	3,345.7		
CT x RM	35	625.5	1.099	.34
Error (CT x RM x S)	175	569.3		
CT x B	7	1,030.1	2.660	.026
Error (CT x B x S)	35	387.3		
RM x B	5	656.1	.616	.69
Error (RM x B x S)	25	1,065.9		
CT x RM x B	35	293.8	.744	.85
Error (CT x RM x B x S)	175	395.1		